Remarks

Claims 20, 22, 25 and 26 stand rejected as being unpatentable over Julich in view of Nagl and Contreras. The rejection states that Julich teaches that sodium hypochlorite (NaOCl) has antiviral activity and Nagl teaches that N-chlorotaurine has bactericidal, fungicidal and vermicidal properties as well as virucidal activity including HSV-1 and 2. The rejection concedes that none of the cited references disclose a combination of NaOCl and N-chlorotaurine for the treatment of lesions and infections generated from periodontitis and herpesvirdiae and the mechanism of action of substantial stimulation of myeloperoxidase activity in the human or animal as claimed. Nevertheless, the rejection finds that it would have been obvious to one skilled in the art to combine NaOCl and N-chlorotaurine to treat lesions and infections generated from herpesviridiase and periodontitis. Allegedly, one skilled in the art would have been motivated to combine the cited references to generate a treatment that has bactericidal, fungicidal and vermicidal properties as well as "an expected additive benefit of antiviral activity." In addition, allegedly, the treatment of lesions and infections generated from periodontitis is obvious because herpesviruses have been implicated in the pathogenesis of human periodontitis as taught by Contreras. The Applicant respectfully requests reconsideration and withdrawal of the obviousness rejection in light of the following.

A proper analysis under Section 103 requires consideration of: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or carry out the claimed process; and (2) whether the prior art would have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991). Both the suggestion and the reasonable expectation of success must be founded in the prior art and not in the applicant's disclosure. *Id.* With respect to the first factor, "[t]he mere fact that references can be combined or modified does not render the

resultant combination obvious unless the prior art also suggests the desirability of the combination." *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990). With respect to the second factor, "focusing on the obviousness of substitutions and differences instead of on the invention as a whole [is] a legally improper way to simply the difficult determination of obviousness." *SeeHybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367 1383 (Fed. Cir. 1986).

First, the Applicant respectfully submits that the prior art would not have suggested to one skilled in the art to make the claimed compound. As conceded by the rejection, none of the cited references suggest combining NaOCl and N-chlorotaurine. In addition, with respect to Claims 25 and 26 specifically, none of the cited references disclose NaOCl or N-chlorotaurine for treating periodontitis or herpesviridiae. Further, there is no implicit suggestion in the prior art or the knowledge generally available to those skilled in the art to combine NaOCl and N-chlorotaurine for treating infections.

The Applicant invites the Examiner's attention to the enclosed letter from Iain L.C. Chapple, Ph.D. and the enclosed article from the October 2007 edition of EFP News. Dr. Chapple is a Professor and Head of Periodontology and Consultant in Restorative Dentistry at the University of Birmingham, School of Dentistry in the UK. He is the Clinical Lead for a hospital specialist periodontal service with a referral base of over 6 million. He also leads a periodontal research team active in the investigation of pathobiological aspects of the host-microbial interface and, novel host-modulation therapies and also point of case assay development. Dr. Chapple has published over one hundred and fifty (150) papers and abstracts and has delivered several keynote lectures at IADR, EuroPerio and BSDR. Dr. Chapple is also a former Scientific Editor of the British Dental Journal and is currently an Editor of the European journal "Periodontal Practice Today" as well as being Editorial Board member of "Periodontology 2000" and the Journals of Clinical Periodontology and

Periodontal Research. Dr. Chapple has received many awards including the Rizzo Research Award of the IADR Periodontal Research Group and the British Society of Periodontology (BSP)'s Sir Wilfred Fish Research Prize. *See* EFP News, October 2007, Vol. 12, No. 1.

As provided in Dr. Chapple's letter, Dr. Chapple is thoroughly familiar with the claimed subject matter. Also according to the letter, there was a long-felt need in the art for a treatment for patients with severe periodontitis that provided consistent results. The Applicant also invites the Examiner's attention to two additional publications included with this response, one by the Applicant, Mainnemare, et al. (2004), pg. 823, and the other by Claffey et al., (2004), which both show this long felt need. In particular, they demonstrate that it had been found that a significant number of severe cases of periodontitis deteriorate over time with non-surgical treatment.

The Applicant's claims are the first to appear to satisfy this need. *See* Chapple Letter & Mainnemare, et al. In fact, Mainnemare states that "low concentrations of [NaOCl and TauCl] are associated with compromises in anti-infection defenses . . . [but] [t]o date, therapeutic use of NaOCl . . . and TauCl solutions in periodontitis has not been considered." *See* Mainnemare, pg. 823. Mainnemare also provides "[t]hus, although never previously recognized, HOCl and TauCl may be of potential benefit as adjunctive therapies for periodontitis patients." *See id.* (Note that NaOCl is HOCl sodium salt.) In other words, even assuming *arguendo* that NaOCl and TauCl were known for antiviral activity and bactericidal, fungicidal and vermicidal properties and virucidal activity, as provided by the rejection, those skilled in the art had not combined them to treat periodontitis. As such, it cannot be found that one skilled in the art would have combined the cited references to meet the claim elements.

Second, the Applicant respectfully submits that the prior art would not have revealed that in so making or carrying out the claimed subject matter, those skilled in the art would have a reasonable

expectation of achieving the technical results which were in fact achieved by the Applicant. Therefore, the Applicant respectfully submits that combining the cited references violates the doctrine of impermissible hindsight reconstruction. *See e.g.*, *In re Geiger*, 815 F.2d 686 (Fed. Cir. 1987).

Periodontitis is known to be a degenerative inflammatory disease generated by Gram negative anaerobic bacteria. Its pathogenesis is characterized from a high similarity with certain autoimmune diseases, such as rheumatoid arthritis. In this regard, the Applicant invites the Examiner's attention to the enclosed article by Moen et al (2003). The specific tissue destructions induced by periodontitis have been shown to be a result of a local dysregulation of the immune system, which is mainly induced by the pathogen itself disturbing the innate system. In this regard, the Applicant invites the Examiner's attention to the enclosed article by Dixon et al. (2004); Gemmel & Seymour, (2004) and Seymour & Taylor, (2004). Thus, although anti-infectious drugs improve periodontitis and herpesviridiae, it is know that the prior anti-infectious drugs available do not consistently, effectively treat the severe form of periodontal diseases (i.e., initial probing depth of periodontal pockets $\geq$ 7 mm). *See* Claffey et al., (2004). These inconsistencies seem to result from the innate immune system, which remains in a dysregulated state. *See* Mainnemare (2004). Similar to periodontal diseases, an acute herpetic attack results from dysregulation of a local immune system, which would generate the cutaneous lesions observed. In this regard, the Applicant invites the Examiner's attention to the enclosed article by Malinovskaya et al., (2000). In fact, this noxious immune regulation would seem to delay or stop the trigger of wound healing.

Therefore, consistent and effective treatment of periodontal disease and herpesviridiae needs to do more than simply provide the antiviral, bactericidal, fungicidal and vermicidal activities of the individual molecules of NaOCl and TauCl. A consistent treatment needs to be capable of:

to do more than simply provide the antiviral, bactericidal, fungicidal and vermicidal activities of the individual molecules of NaOCl and TauCl. A consistent treatment needs to be capable of:

1. Destroying infectious agents that are responsible for the diseases,
2. Neutralizing the harmful molecules released from pathogens before and during their remove,
3. Regulating the physiology of the immune system, and
4. Healing tissue.

The Applicant's claims do all of the foregoing. Thus, the Applicant's claimed composition has additional properties beyond the anti-infectious properties outlined in the rejection. In particular, these properties are included in new Claim 27. Support for the elements of Claim 27 is found at paragraphs [0016], [0032] and [0077]. These properties result from both (i) the complementarities and (ii) the synergic effects generated from the two active agents. As a result, in his letter, Dr. Chapple states that "[t]he combination of sodium hypochlorite (40 nM) and taurine N-monochloramine (300 μM) is novel and may provide ***synergistic benefits*** when used topically as an adjunct to non-surgical periodontal therapy." *See* Chapple Letter (emphasis added). In addition, Mainnemare et al. provides "[NaOCl and TauCl] act synergistically to modulate the inflammatory response." *See* Mainnemare et al., Abstract. In other words, the combination of the aforementioned molecules yields unforeseen benefits. As provided above, one skilled in the art would not have reasonably expected such success as evidenced by the fact that no one had combined NaOCl and TauCl despite the problems with treating periodontitis and herpes, known in the art. For the foregoing reasons, the Applicant respectfully submits that one skilled in the art would not have been motivated to combine the cited references.

Nevertheless, even assuming *arguendo* that there is some motivation to combine the references, for a combination of known elements to be non-obvious, the combination of elements

must do more than function as they would individually. Here, provided above, the combination of NaOCl and N-chlorotaurine does more than they would individually, i.e., simply kill viruses, bacteria, etc., which is provided by the rejection as the only motivation for combining these compounds. The combination of NaOCl and N-chlorotaurine destroys infectious agents that are responsible for the disease, neutralizes the harmful molecules released from pathogens before and during their removal, regulates the immune system and promotes and induces tissue healing. The cited art does not disclose these functions. Withdrawal of the rejection is respectfully requested.

In light of the foregoing, the Applicant respectfully submits that the entire application is now in condition for allowance, which is respectfully requested.

Respectfully submitted,

T. Daniel Christenbury
Reg. No. 31,750
Attorney for Applicant

TDC/vp
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Thursday, 14 October 2004

I, the undersigned,

Professor Iain L. C. Chapple – BDS, FDSRCPS (Glas), FDSRCS (Edin), PhD

declare that I am aware of the work of Dr Arnaud Mainnemare and read and discussed his research poster at the 4th European Conference of Periodontology (Berlin Germany).

His preliminary data demonstrated that combining sodium hypochlorite and taurine N-monochloramine at low concentrations appears to have potential as a novel topical anti-inflammatory adjunctive therapy for managing chronic periodontal disease. It will be interesting to see follow up data from controlled studies using this novel combination.

Whilst non-surgical periodontal therapy is efficacious in the majority of patients with mild to moderate periodontitis, those with severe disease at several sites respond less predictably. Given the widely accepted role of an aberrant host inflammatory-immune response in causing the majority of tissue destruction in periodontitis, host-modulating therapies have a key role to play as therapeutic adjuncts in the future.

There is some data in the literature on the role of "concentrated" sodium hypochlorite solutions improving clinical outcomes in periodontal therapy, but data on attachment gain (the gold standard outcome measure) is limited and unconvincing.

The combination of sodium hypochlorite (40mM) and taurine N-monochloramine (300μM) is novel and may provide synergistic benefits, when used topically as an adjunct to non-surgical periodontal therapy. Further investigations of the dynamic interactions of these two molecules and their effects upon the periodontal connective tissues and inflammatory-immune response may lead to new approaches to periodontal therapy, based upon modulation of the host response. I am unaware of this combination of molecules being used for this purpose to date, other than by Dr Mainnemare and colleagues.

I Chapple

Professor Iain L. C. Chapple

Vol. 12, No 1, October 2007

EFP News

www.efp.net/EFP Newsletter

>>>>General Assembly>>>>

Florence, Italy • 24 February, 2007

The last General Assembly was held in Florence on Saturday 24 February, 2007.

Members of the Executive Committee and the General Assembly met at Convitto della Calza a conference centre in the historical centre of Florence. Originally a 14th-century cloister, it is frescoed with masterpieces such as Francibigio's "Jesus Last Supper". The culture and tradition of this fabulous ambiance offered the appropriate atmosphere for collective work, which was greatly enhanced by the excellent organization of the Italian Society of Periodontology.

Among numerous items on the Agenda were: Reports from the President, Secretary General, the Treasurer; and the Chairmen of the standing committees.

The new Secretary General Dr. Baehni presented an action list for his mandate. He focused on: improving management aspects (legal, accounting, administrative); improving communication with the national societies (keeping them informed regularly on EFP current activities); meeting with the boards of the national societies; changing the format of the GA to allow more participation from national societies (providing reports, documents in advance); developing undergraduate as well as continuing education; developing and implementing strategies to increase visibility of the EFP; developing awareness and preventive campaigns addressed to the public, working in a partnership program with industry.

Many issues were addressed, discussed and approved.

In particular, the significantly prominent items at this General Assembly were the Strategic Planning process which the EFP has undertaken during the last year with the guidance of Prospectus, a specialist healthcare consulting company. The vision, the strategic objectives and action plan of each objective identified in the process were presented. Following this presentation, Prospectus consultants facilitated a short session with the General Assembly to collect some initial feedback on the strategy. It was agreed that the Presidents and EFP delegates bring the discussion of the Strategic Planning process to their own national societies, collect the feedback of their members on the overall impression of the strategic plan, the vision and the strategic objectives and send a written report on the Strategy to the EFP. The feedback from all of the societies will be summarised and presented to the Executive Committee for consideration at the September meeting. The feedback will be studied and amendments will be made to the strategy if required. The revised plan will be presented to the General Assembly for approval in February 2008.

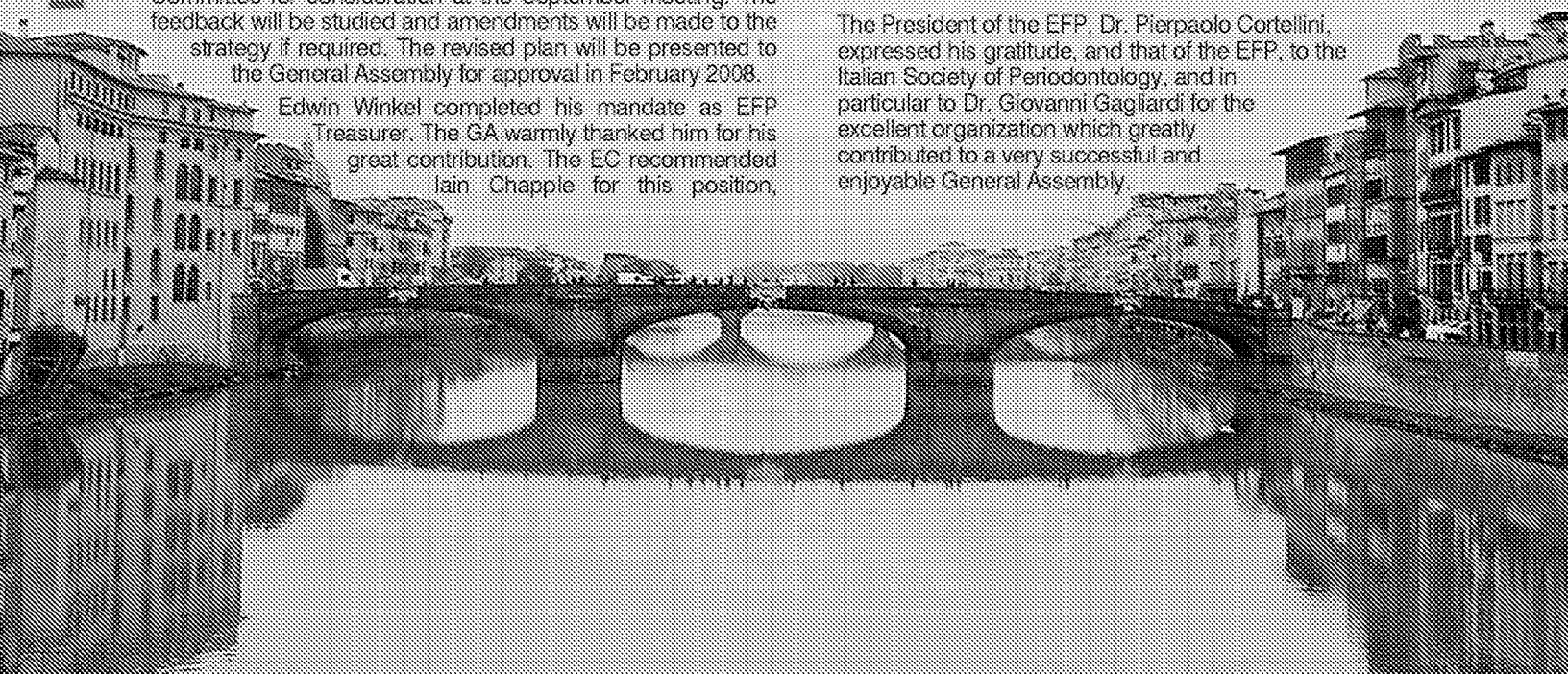
Edwin Winkel completed his mandate as EFP Treasurer. The GA warmly thanked him for his great contribution. The EC recommended Iain Chapple for this position, stressing his expertise as past-treasurer of the British Society of Periodontology. The proposal was unanimously approved by the General Assembly.

Executive committee at the Franciabigio Hall.

Israeli Society of Periodontology was accepted as full member of the EFP. Israel belongs to the 53 countries of the Europe zone according to WHO.

At the end of the General Assembly, President Pierpaolo Cortellini offered a token of our Federations deep appreciation and gratitude, to Morten Klepp and Edwin Winkel for their valuable contribution to the success of the Federation as tireless and highly knowledgeable Members of the EFP Committee.

The President of the EFP, Dr. Pierpaolo Cortellini, expressed his gratitude, and that of the EFP, to the Italian Society of Periodontology, and in particular to Dr. Giovanni Gagliardi for the excellent organization which greatly contributed to a very successful and enjoyable General Assembly.



message from the president

Dear friends and colleagues,

As the newly elected President of the European Federation of Periodontology I want to thank you all for your support, confidence and friendship.

I will try to serve the federation in the continuity of my predecessors in providing intellectual input but also strengthening the efforts in order to fulfil our mission to be the driving force of Periodontology in Europe. I am very pleased, that I have a group of fantastic friends around, who are all inspired by the same vision, which strengthens any effort.

As you may have recognized the EFP is currently undertaking major efforts to improve service and support for all member societies. Based on your feedback, we will now refine our strategy.

One of the major goals is the official recognition of Periodontology as a specialty on the European level. This is a common task and I want to encourage everybody to use all influence, to give us the opportunity to achieve this in short time.

Exciting new research results and research initiatives will help us on this way and are very much welcome. So please keep us updated on the developments in your countries, so that we can circulate the information and help each other. We are looking forward to another Ittingen Workshop which, under the umbrella of the EFP, will again be organized by Klaus Lang. I am pleased and very thankful, that Klaus has taken the burden of the organization for another time and I am sure that this will create new knowledge and contribute to the strength of our federation.

Everybody is aware that in some cases periodontitis is cured by tooth extraction and implant placement. We must educate our young colleagues, that in many cases natural teeth are the better alternative and any efforts should be undertaken to keep the own dentition. Host response and inflammation are not excluding implants.

The voice of the European Federation is highly respected and together with our friends in the American Academy we will raise it in order to achieve our commitment, which is in the constitution of our federation to improve periodontal health in Europe and on a world-wide level.

I hope very much, that during my presidency we can make further progress and follow the path, which has been led by my dear friends and previous presidents.

Joerg Meyle >>> President of the European Federation of Periodontology

>>>Recognition of Periodontology as a Speciality

Periodontology is currently considered a formal dental speciality in 11 countries belonging to the EU however it lacks this legal status in the other 14 countries. EFP has approached the EU to rectify this situation and obtain acknowledgement throughout the EU.

The position paper Periodontology as a recognized dental speciality in Europe, by Mariano Sanz, Ubele van der Velden, Daniel van Steenberghe, and Pierre Baehni published in JCP in June 2006 was a very good initiative to provide evidence for the need for a recognized specialty in Periodontology at European level. This paper focused on both the educational and professional perspective, with the hope of providing discussions that may contribute to facilitate its legal establishment as a new dental speciality in Europe. Uros Skaleric, chairman of the «ad hoc committee for the recognition of Periodontology as a Speciality», reported on the actions taken on the part of the EFP towards the EU and the Council of European Chief Dental Officers (CECDO).

The response from the European Commission Internal Market and Services Regulated Professions was that European Directives still provide for automatic recognition for the two specialities in orthodontics and oral surgery and it maintains the procedure for the inclusion of additional specialities in the system of automatic recognition. This means that there is no simplified procedure (like the one applicable to medical specialities) to introduce additional dental specialities in the system of automatic recognition of dental specialities. The only possible procedure to do so would be to amend the Directive itself through a new legislative co-decision procedure (i.e. the same procedure as for its adoption which means adoption by the European Parliament and by the Council of Ministers).

The response of the CECDO was that they fully agree and support official recognition of Periodontology as a dental speciality. However, since this relates to the organisation of the delivery of care of each country, the official recognition of individual dental specialities should be the responsibility for each country to decide taking into account its specific national circumstances.

Therefore, Dr. Skaleric and Dr. Sanz recommended that national societies pursue the process of recognition of the speciality at national level. It is crucial to design a good strategy. Mariano Sanz reported about the work done with other specialities to produce a document to approach governments.

message from the editor

Dear EFP Members,

Under the guidance of Prospectus, a professional consulting company, significant progress in the Strategic Planning of the EFP has been achieved this year, and all the committees have been actively contributing to the advancement of the Federation. This close collaboration strengthens our Federation and makes sure that the voice of European Periodontology is strong and highly respected.

Although the recognition of the periodontal speciality is a slow procedure, a positive step forward was made with the publication of the position paper Periodontology as a recognized Dental Speciality in Europe, prepared by Mariano Sanz, Ubele van der Velden, Daniel van Steenberghe, and Pierre Baehni. The paper, which was published by JCP in June 2006, clearly identifies the need for a recognized specialty in Periodontology and we are confident it will contribute to the facilitation of Periodontologys legal establishment as a new dental speciality throughout Europe.

Further efforts are also being made towards the development and implementation of a strategy to achieve this important goal.

The EFP News aims to serve you by keeping you all up-to-date on the efforts and projects being undertaken by the Federation. Another important task of this newsletter is to inform all our members about future events in the fields of periodontology, implant dentistry and other perio related issues.

The continuous contributions of news and articles from the National Societies are vital for the dissemination of information to all our members. I would like to thank all those colleagues who have spared the time and effort to contribute in making the EFP news interesting, informative and pleasurable to read. As always, we welcome your news, views and suggestions so that the EFP News can continue to keep you all advised of what is happening in our Federation.

The future is looking very bright indeed.....

Joanna Kamma >>>Editor of the EFP News

Changes in the EFP Treasury

Edwin Winkel stepping down as Treasurer of the EFP >>>>>>

Treasurer from 2001, Edwin has been one of the key players in the development of the EFP. His participation commenced at the Paris Euro Disney meeting in June 1992 when he was proposed as a Member of the EuroPerio1 Organising Committee and was appointed Treasurer of EuroPerio1 and 2. Since then Edwin has selflessly devoted his time and energy towards the advancement of the Federation in many key areas over the years.

Edwin has actively participated in crucial EFP issues, particularly: the organisation of the EuroPerio Congresses, and conducting all negotiations with the Brussels lawyers for legalisation of the EFP as an AISBL (European non-profit making scientific association).

Edwins most recent contributions include the founding of the EEIG (European Economic Interest Grouping); Legalisation pertaining to the EFP and valuable input into securing financial stability for the Federation.

Edwin is still actively contributing more and more to the EFP in his role as Treasurer for EuroPerio6, which is scheduled to take place in Stockholm 4-6 June 2009.

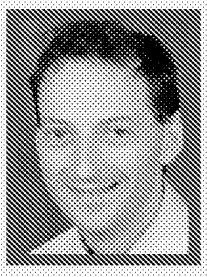
Apart from his substantial involvement with the EFP as Treasurer, Edwin holds many other important posts. He has been Clinical Instructor and Lecturer on Post Graduate courses on Periodontology at the Academic Centre for Dentistry (ACTA) (1986-1994) in Amsterdam, State Examiner at the School for Dental Hygienists (1989-1994), in Amsterdam. Edwin has served as President and Treasurer of the Dutch Society of Periodontology, was Founder and a Board Member of the European Association for Osseointegration, a Member of the Advisory Board of the Dutch Society of Periodontology, and a Member of the Past Presidents Committee of the Dutch Society of Periodontology to name a few. Currently he is associate professor at the Academic Centre Oral Health, University Medical Centre Groningen.

Edwin has lectured extensively at a national and international level about systemic antibiotics, oral malodour and implant dentistry. He is author of a great many publications and is Editorial Board member of many periodontal journals.

Edwin Winkel, on behalf of the European Federation of Periodontology and all your friends and colleagues who have enjoyed working with you over the years thank you for your fantastic contribution to the administration and for your dedication, friendship and support over the years; you have worked so diligently for the EFP.

While we are sad to bid farewell to Edwin, his post is being filled by a worthy successor, Iain Chapple, who will be devoting his efforts to the continuation of Edwins fantastic achievements.

>>>>>> Iain Chapple appointed as new Treasurer



Iain Chapple is currently Professor and Head of Periodontology and Consultant in Restorative Dentistry at Birmingham Dental School and Hospital. He is the clinical lead for a hospital specialist periodontal service with a referral base of over 6 million. He also leads a periodontal research team active in the investigation of pathobiological aspects of the host-microbial interface and, novel host-modulation therapies and also point of case assay development. Iain has published over 150 full papers and abstracts and has delivered several keynote lectures at IADR, EuroPerio and BSDR.

Iain is a former Scientific Editor of the British Dental Journal and is currently an Editor of the European journal «Periodontal Practice Today» as well as being Editorial Board member of Periodontology 2000 and the Journals of Clinical Periodontology and Periodontal Research. He is the Periodontal Editor of a series of 7 textbooks by Quintessence and the author of 6 of these texts. Iain was awarded the Rizzo Research Award of the IADR Periodontal Research Group and the British Society of Periodontology (BSP)'s Sir Wilfred Fish Research Prize.

Iains management profile includes being former was President of the International Association for Dental Research (IADR) Periodontal Research Group (2006-2007), former Chairman of the British Society for Dental Research (BSDR) Periodontal Research Group, Chair of the BSDR's Strategic Review Committee for Oral and Dental Research and Member of the UK Department of Health's working group on Dentists with Special Interests in Periodontology in the UK. He has been a council member of the BSP since 1994 and former Honorary Treasurer and founding member of the BSP's Education and Conference Committees.

Please submit your articles for the next EFP News before 31 January 2008 to:

Joanna J. Kamma • Editor - EFP News
6-8 Freattidos St., GR-185 37 PIRAEUS, GREECE, Fax: + 30 210 4525 935
e-mail: joannakamma@gmail.com

Prizes & awards PRIZES

Italian Society of Periodontology SIdP

The main mission of the Italian Society of Periodontology is to promote the prevention and the diagnosis of diseases of the parodontal tissues. Furthermore the functional and aesthetic rehabilitation, including through implant procedures, is an important goal of our therapies as improvement of the way of life. SidP is devoted to promote scientific research and continuing education programmes in Periodontology, Implantology and in related medical-biological disciplines by means of grant and research prize competions.

Henry M. Goldman Prize

Papers may relate to original basic, laboratory or clinical research (clinical trials or
innovative surgical techniques) provided they pertain to all aspects of periodontal and
implant biology and clinical practice.
The two winners, one for basic research and one for clinical research, will be awarded the
"Henry M. Goldman" prize and 2000€ in cash.

Scolarship for two years programme

The winner will be to participate to the scientific research programme for two years in a prestigious university. The selected venue for the next period 2008/2009 will be the University of Madrid.

SIdP Prize competition for a degree thesis about Periodontology and Implantology

The prize will be awarded every year at the meeting of the Teaching College in Dentistry. The scientific commission will select five nominees who will participate in the final competition. The finalists will present their works in a 20 minutes oral presentation. The winner will receive a monetary prize award of 1000€ together with the publication on the web site of SIdP.

Michele Cagidiaco Prize

The Italian Society of Periodontology is pleased to announce the Michele Cagidiaco Prize competion dedicated to students and graduates in Dental Hygiene.
The prize is reserved to the best poster presentation and will be awarded at the SidP National Congress 6-8 March 2008.
The winner will receive a monetary prize award of 1000€.
Information at *info@sidp.it* or at the web site *www.sidp.it*.

EFP Prize for Graduate Students in EFP approved Graduate Programs EFP

The EFP Research Committee calls for candidates in the Graduate Research Prize competition. The prize is awarded once a year and is open to all graduate students of the EFP approved graduate programs.

The research work must have been published between January and December of 2007 in the *Journal of Clinical Periodontology*, or in another internationally recognized peer reviewed journal.

The deadline for manuscript submission is 2nd January, 2008.

Original research articles should be sent to the

EFP European Coordinator, Mónica Guinea

Spanish Society of Periodontology
Antonio Lopez Aguado 4 - bajo dcha.
SP - 28029 Madrid
SPAIN
Tel: +34-91-3142715
Fax: +34-91-3235745
Email: monica@efp.net

The winners will receive a monetary prize award of 1.000€ for the 1st prize and 500€ for the 2nd prize together with an Award Certificate.

New Boards from the National Societies

Israeli Society of Periodontology and Osseointegration

President: Vig Shlomo
Past President: Hezchiel Dorfan
President Elect: Ferzkin Bonfman
Secretary: Shlomo Alon
Treasurer: Eran Friedwald
EFP Representative:
Moshe Goldstein
Webmaster: Eyal Yonezat

Spanish Society of Periodontology

President: Juan Blanco
Vice President: Nuria Vallcorba
Secretary: David Herrera
EFP Delegate: Mariano Sanz
Vocals: Adrian Guerrero, Antonio Liñares, Hector Juan Rodriguez Casanovas, Webmaster Francisco J. Enrile

Hellenic Society of Periodontology

President: Antonis Konstantinidis
Vice President: Ioannis Vrotsos
General Secretary: Georgios Baltas
Treasurer: Nikos Kalaitzakis
Members: Maria Theofanatos, Christos Katsadi, Anastasia Golz
Non voting members: Vasilios Panis, Eli Smirlich
EFP Representative: Andreas Parashis
Webmaster: Nikos Kalaitzakis

1995

EFP meetings were held in Gothenburg, Sweden, 8 June 1995 and in Istanbul, Turkey, 8-10 December 1995.

The Gothenburg meeting coincided with the 4th North Sea Conference. During the June meeting, it was agreed that the Committee for Promotion of Research consisting of Gil Alcoforado and Michel Brecx was assigned to investigate the research grants that may be available in Europe, and the committees which could be approached.

A logo for the EFP was agreed upon from the three designs suggested by the Scandinavian, Swiss and British representatives. This new logo was incorporated into the EuroPerio 2 logo. The logo was also sent to Munksgaard Publishers who then modified the front page of the *Journal of Clinical Periodontology* accordingly.

At the December meeting the membership of the Post-Graduate Committee for the inspection of the Post-Graduate Programmes was finalised. It was confirmed that Ubele van de Velden be appointed as Chairman of the Post-Graduate Committee (Pierre Baehni, Lavinia Flores de Jacoby, Mariano Sanz). Prof. Van de Velden advised that he would prepare a comprehensive questionnaire appropriate for EFP use in evaluation of Post-Graduate Programmes.

On setting criteria for membership of the EFP, it was also agreed that a Sub-Committee be formed consisting of Jean Louis Giovannoli, Mariano Sanz, Pierre Baehni, Lavinia Flores de Jacoby, Ubele Van der Velden, David Hillam to examine all aspects of the process by which new countries could be admitted to membership of the EFP.

President
Lavinia Flores de Jacoby

President Elect
Ubele van der Velden

Immediate Past President
Pierre Baehni

General Secretary
David Hillam

Treasurer
Michel Brecx

The first EFP Directory of Periodontists and Dentists with a special interest in Periodontology was completed and presented by Lavinia Flores de Jacoby. This directory would facilitate the referral of patients with periodontal problems and it was agreed that the list should be updated in November of each year.

Submissions had been received from the British, German and Swiss societies for EuroPerio 3 to be held in Glasgow, Munich and Geneva. Following short presentations from Isobel Madden, Joerg Meyle and Pierre Baehni, the proposal of the Swiss Society of Periodontology was voted to host EuroPerio 3 in Geneva, 7-10 June, 2000. Pierre Baehni was appointed Chairman of EuroPerio 3, Ubele van der Velden the Scientific Chairman and Jean Louis Giovannoli the congress Treasurer.

To be continued...

Joanna Kamma

>>>>6th European Workshop on Periodontology

>>>>Ittingen, 2-6 February 2008

The 6th European Workshop on Periodontology, which is organized by the European Academy of Periodontology Workshop Committee, will be held from 2-6 February 2008 in Charterhouse at Ittingen, Switzerland.

The workshop is supported by an educational grant of Straumann. The Scientific Programme Committee comprises Niklaus Lang, Chair, Jan Lindhe, Mariano Sanz, Maurizio Tonetti and Denis F. Kinane. The main theme of the workshop is Innovation and Periodontal Practice comprising 5 working groups.

The following topics will be addressed:

- Innovations in non-surgical periodontal therapy
- Periodontal tissue engineering and regeneration
- Critical issues in bone regeneration and implant therapy
- Peri-implant infections
- Periodontal diseases and health

The proceedings of the 6th European Workshop on Periodontology 2008 will be published by Blackwell Munksgaard as a Supplement to the Journal of Clinical Periodontology

Students graduated from EFP approved graduate programmes in 2006

Academic Centre for Dentistry Amsterdam: J. Stoeken, S. Tjoa
Catholic University of Leuven: A. Marcelo, K. Michels, W. Teughels
University of Nijmegen: H. Nguyen, D. Oortgiesen, B. Weinstock
University of Jonkoping: D. Tomic, I. Loggner Graff, A. Mohei
Fjellsson
University Complutense of Madrid: Ana Echeverria, Jorge Ferrus, Sergio Morante, Fabio Vignoletti
University of Göteborg: Catharina Bandmaier, Eleni Chyta, Kirykos Martakis, Davide Sala, Alberto Turri, Antonios Zampelis

Annual Meeting of the Austrian Society of Periodontology St. Wolfgang, 19 - 21 April 2007

This year, more than 600 doctors and assistants greeted the President of the GP, Dr. W. Müller. The consistently growing number of members proves the unbroken popularity of this major event.

The motto assigned to the 16th Annual Meeting of the Austrian Society of Periodontology by the coordinators of the meeting Dr. W. Wadsak and Dr. K. Charvat was Periodontology pure.

At 21 workshops and parallel lectures on various subjects and areas of knowledge, all attendees were able to acquire valuable knowledge. The meeting was organized with extensive effort. Opportunities for practical training were offered in a large number of courses, such as practical improvements in restorative periodontal surgery, in keeping with the state of the art. Advancements in debridement by the use of manual and sonic instruments, surgical assistance, photographic documentation and many other subjects were also presented.

Renowned specialists from Austria, Germany, Switzerland and Belgium lectured simultaneously in several halls. To cite a few of these stellar names in their respective fields: Prof. Dr. M. Quirynen, Leuwen (B), Prof. Dr. U. P. Saxer, Zürich, Prof. Dr. J. Meyle, Prof. Dr. A. Mombelli, Prof. Dr. Stefan Zimmer, Düsseldorf, Priv. Doz. Dr. M. Christgau, Düsseldorf, Dr. W. Bengel, Bensheim.

The spectrum of offered subjects included introduction to dental prophylaxis, periodontal surgery, associations with general medicine, antibiotic treatment, cessation of smoking, care of patients undergoing chemotherapy and those with Alzheimers disease to name a few.

Live operations were a special highlight this year as well. Dr. Wachtel of Munich demonstrated, in a fully equipped in-house operating room, several procedures including his newly modified tunnel technique of recession coverage, and also explained the procedure. In the coming issues we will inform our members about new aspects and details of these lectures.

The main focus of the traditional and highly popular social evening at the Circus Circus was the Million Zähnt Cent Show. The professional master of ceremonies was Dr. A. Mory. The winners were pleasantly surprised by the significant sums of money awarded as prizes.

Those who were not inclined to spend their breaks under the warming sunshine of the Wolfgangsee and its surrounding mountains, still coated with a gentle layer of snow, could use their breaks to inform themselves about the most innovations in dental products at the extensive exhibition that accompanied the meeting.

At the ÖGP stand one could obtain information about the European Dental Association (EDA) and its options of specialization.

A new feature at the Medical University of Vienna is a postgraduate University course culminating in a Master of Science degree, which can be attended parallel to employment and will be offered from the summer semester of 2008 onward. The Austrian Society of Periodontology promotes and supports this additional training course and would like to encourage its own members to avail themselves of this opportunity.

For further information go to: *www. paromaster.eu or www.oegp.at*

News of British Society of Periodontology in 2007

- It is with great sadness that the BSP announces the passing of 3 longstanding members of the Society this year. Dr John Zamet, BSP President 1977-78 and Honorary Member, Dr Dick Veldkamp who was conferred honorary membership in 1983 and Dr Stuart McKenzie, BSP President 1968-69. Full obituaries will appear in the next issue of BSP News this winter.
- The BSP has undertaken a major market research exercise into undergraduates and newly qualified dentists attitude towards periodontics as a specialisation and this project is now nearing completion. Results will be published in due course.
- The British Dental Hygienists Association has now changed its title to The British Society of Dental Hygiene and Therapy (BSDHT), reflecting changes in hygienist/therapy training in the UK. (www.bsdht.org.uk).
- The next issue of BSP News will be available in January 2008.

Philip Dwer
BSP News Editor

Spring Scientific Meeting of the British Society of Periodontology

The BSP meeting held on 10 & 11 May 2007 in the superb Edinburgh International Conference Centre stands out in memory as one of the great BSP meetings. The theme for the meeting was Periodontology the Later Years. Professor Panos Papapanou (Columbia University, New York) gave the first presentation on the epidemiology of periodontitis in the elderly, emphasising the periodontal implications of aging populations retaining more teeth. Dr Jonathan Bodansky (Leeds General Infirmary) gave an authoritative talk on medical aspects of diabetes and delivered the stark take-home message that we should all be aware of the undiagnosed diabetic patient sitting in our dental chair. The epidemiological research linking periodontal disease and diabetes was then reviewed by Professor Phoebus Madianos (University of Athens), and this was followed by a lecture from Professor Evanthia Lalla (Columbia University, New York) that considered the mechanistic links between these two conditions.

Professor Papapanou then returned to give a stimulating discussion on risk factors for aggressive periodontitis, including a thought provoking assessment of the difficulties that we face when trying to interpret the diagnostic criteria for aggressive periodontitis as presented in the 1999 World Workshop on periodontal disease classification. The effect of sex hormones on the periodontal tissues was then reviewed by Dr Philip Preshaw (Newcastle University), who identified the dearth of quality research in this area. Professor Robin Seymour (Newcastle University) gave the final presentation on the first day of the conference: a review of the problem of drug-induced gingival overgrowth, in which he emphasised the need for good communication with our medical colleagues when managing patients taking these drugs.

The conference dinner was held at the spectacular Museum of Scotland in the city. The second day opened with Professor Angus Walls (Newcastle University) who delivered a fascinating lecture on restorative considerations in the elderly, emphasising the importance of prevention and planning for failure. He raised the pertinent point that the definition of elderly depends on your own age, and is generally reckoned to mean someone who is at least 15 years older than you are!

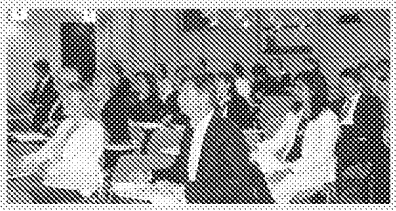
Professor Roy Taylor (Newcastle University) then gave a thought provoking lecture intriguingly entitled Metabolic syndrome: badge of success for the hunter-gatherer? This excellent talk ranged from ancient Egypt, where geese were force fed grain to make foie gras in a very similar manner to today (with Professor Taylor asking the question of whether modern humans, with their excess food intake, are in fact very similar to these force fed geese), to present day genetic analyses to identify genes that increase risk for diabetes, to finally presenting a solution to combat both the current diabetes epidemic and problems of carbon emissions: namely transport (i.e. we should all be walking or cycling much more rather than driving).

The links between diet and periodontal disease were then explored by Dr Paula Moynihan (Newcastle University), and associations between periodontal disease and cardiovascular disease and obesity were reviewed by Dr Thomas Dietrich (Boston University). Dr Mike Milward (Birmingham University) reviewed the impact of age on periodontal inflammatory responses, and the final lecture of the conference was delivered with typical humour and authority by Professor Martin Addy (Bristol University) who considered the difficulties of treatment planning in older patients. These lectures concluded a really outstanding BSP conference in an outstanding conference venue in a fabulous location, with a sparkling array of excellent, internationally-renowned speakers.

Philip Dwer
BSP News Editor

EuroPerio 6

Stockholm, Sweden, 4 - 6 June 2009

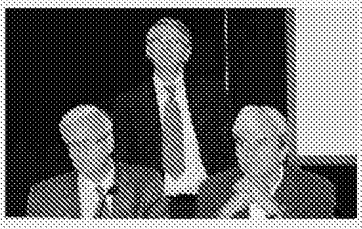


Industry Partners during the gathering.

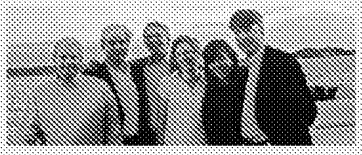
EuroPerio 6- Industry gathering in Stockholm

1st June 2007 The EuroPerio 6 committee presented the congress organisational plan to the industry in Stockholm. More than 50 delegates from Swedish and International companies attended this gathering. Dr. Stefan Renvert presented the layout of the congress and Dr. Sandro Cortellini the highlights of the scientific program. Their presentations were followed by Dr. Edwin Winkel who presented the sponsors dossier and Dr. Ola Norderyd the promotional plan in Sweden. After this, the delegates visited the venue guided by the committee and representatives of the venue. Finally, the delegates attended a lunch offered by the EFP in Rica Talk Hotel. The feedback was extremely positive. The participants expressed great willingness to participate actively in EuroPerio 6.

Saturday 2nd June the committee met to work on the scientific program, promotional plan, commercial exhibition and sponsorship program. The committee wants to thank all you in the national societies for your contributions to the scientific program. Invited by the Scandinavian Society of Periodontology, the committee attended a reception in the City Hall and a cruise in the Stockholm Archipelago.



Edwin Winkel, Stefan Renvert, Pierpaolo Cortellini.



Edwin Winkel, Pierpaolo Cortellini, Stefan Renvert, Helena Renvert, Monica Guinea, Ola Norderyd.

Dr. Stefan Renvert / EuroPerio 6 Chairman

EuroPerio 6 Organising Committee

Chairman: Stefan Renvert
Scientific Chairman: Pierpaolo Cortellini
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Chairman: Ola Norderyd
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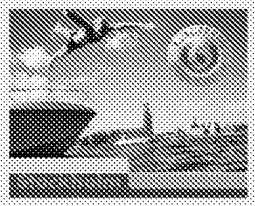

American Academy of Periodontology (AAP) International Membership - *Join Us!*

The AAP offers International membership to any dentists residing outside of the United States and its territories who are interested in the art and science of periodontology and who are members of a recognized national dental association in their country.

Membership applications are accepted throughout the year. You can complete a membership application online at: **http://www.perio.org/about/who.html#applications**. Enter promotion code: **EFPN07** and your $50 initiation fee will be waived.

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- The monthly ***Journal of Periodontology*** is mailed to members and also available online. Members have instant access to the searchable online ***Journal*** where the current year's articles with full text and graphics can be found as well as articles from the past eight years.
- The Academy's ***Membership Directory*** is also online and updated daily so you always have the most current contact information available.
- **Perio.org News**, a monthly e-mail message filled with brief reports on Academy activities and important dates and deadlines, is sent to all members who have e-mail addresses on file at the Academy.
- Every year the Academy holds an **Annual Meeting** that attracts over 5,000 attendees; and members receive reduced rates.
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www.perio.org

The next Annual Meeting is scheduled for September 6-9, 2008 in Seattle, WA.

Perio Societies Meetings and Conferences Calendar

16-17 November 2007
Dental Pain Society Conference 2007
Joint meeting of the British Society of Periodontology, British Endodontic Society, British Society for Restorative Dentistry and the British Society for the Study of Prosthetic Dentistry
Venue: International Convention Centre, Birmingham, UK
Title: BSP: Success In Periodontics
BES:Definitive Endodontic Therapy: Essential Technique Principles
BSRD:Aesthetics Vs Cosmetics
BSSPD: Removable But Not Yet Redundant
Joint day :Treatment Planning - Assessment Of The Mutilated Tooth/Dentition.
Information: www.pandental2007.org

6-8 March 2008
XV National Congress of the Italian Society of Periodontology
Venue: Bologna, Italy
Title: Metodologia in Parodontologia ed Implantologia
Information: www.sidp.it

26-28 March 2008
The 5th International Congress on Perio-Prosthesis in Israel
The Israel Periodontal and Osseointegration Society
The Israel Prosthodontic Society
Venue: Tel Aviv
Information: retzkin1@bezeqint.net

3-5 April 2008
Annual Meeting of the Austrian Society of Periodontology
Venue: Scalaria, St. Wolfgang, Austria
Information: www.oegp.at

13-15 April 2008
Spring Meeting of the British Society of Periodontology
Venue: West Road Concert hall, Cambridge, UK
Title: Confusion, Confounders and Compliance
Information: www.bsperio.org.uk

22-23 May 2008
42 Annual meeting of the Spanish Society of Periodontology
Venue: Palacio Euskalduna - Bilbao Spain
Information: www.sepa.es

5-7 June 2008
Meeting of the French and the Belgian Societies of Periodontology
Venue: La grande motte
Title: Engraved in the time
Information: www.sfparo.org

Periophernalia

1-3 February 2008
International Bone Symposium
Venue: JW Mariott Las Vegas Resort & Spa, Las Vegas, USA
Title: International Bone Symposium
Information: service@quintbook.com

28 February - 1 March 2008
23rd Annual Meeting of the Academy of Osseointegration, American Academy of Periodontology, American Association of Oral and Maxillofacial Surgeons, and American College of Prosthodontists
Venue: Boston, Massachusetts, USA
Title: Implant Dentistry: A Trip up the Implant
Information: www.osseo.org, www.perio.org

2-5 April 2008
Annual meeting of AADR
Venue: Hilton Anatole Hotel, Dallas, Texas, USA
Information: www.iadr.org

2-5 July 2008
86th General Session & Exhibition of the IADR
Venue: Metro Toronto Convention Centre, Canada
Information: www.iadr.org

EFP's new web site will be shortly available at www.efp.net

- Goal
- Mission statement
- Letter of the President
- EFP Officers
- EFP Committees
- EFP Office
- EFP Bylaws
- EFP Group
- How to become a member of the EFP

About the EFP

In August 1987 the first activities aiming to the creation of a platform for the co-ordination and the improvement in the field of periodontology started.

During the 4th meeting in May 1990 in Maastricht, which was presided by U. van der Velden, a constitution including the rules of procedure for the EFP was put up and discussed. It was agreed on the organization of a European Meeting in May 1994 in Paris.

Besides three very successful conferences (Europerio 2 with more than 3500 participants and Europerio 3 with 4000) the EFP succeeded in the invention of standardized European guidelines for student and postgraduate education in the field of periodontology.

Upcoming events

30-08 Dutch congress of periodontology
30-08 EFP Meeting

Latest news

08-08 Europerio 2009 in Stockholm
04-08 Health situation in Europe
10-08 1st Research Prize
08-08 European Workshop on Periodontology
20-04 The Jaccard EFP Research Prize

Sponsors

CONCISE REVIEW
Biological

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Hypochlorous Acid and Taurine-N-Monochloramine in Periodontal Diseases

ABSTRACT

Chronic periodontitis is a multi-factorial disease involving anaerobic bacteria and the generation of an inflammatory response, including the production of metalloproteinases, pro-inflammatory cytokines, and eicosanoids. Hypochlorous acid (HOCl) and taurine-N-monochloramine (TauCl) are the end-products of the neutrophilic polymorphonuclear leukocyte (PMN) respiratory burst. They act synergistically to modulate the inflammatory response. In the extracellular environment, HOCl and TauCl may directly neutralize interleukin 6 (IL-6) and several metalloproteinases, while HOCl increases the capacity of α_2-macroglobulin to bind Tumor Necrosis Factor-alpha, IL-2, and IL-6, and facilitates the release of various growth factors. TauCl inhibits the production of inflammatory mediators, prostaglandins, and nitric oxide. HOCl activates tyrosine kinase signaling cascades, generating an increase in the production of extracellular matrix components, growth factors, and inflammatory mediators. Thus, HOCl and TauCl appear to play a crucial role in the periodontal inflammatory process. Taken together, these findings may offer opportunities for the development of novel host-modulating therapies for the treatment of periodontitis.

KEY WORDS: periodontitis, hypochlorous acid (HOCl), taurine-N-monochloramine (TauCl), cytokine, inflammation, healing.

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A supplemental appendix to this article is published electronically only at http://www.dentalresearch.org.

INTRODUCTION

In an aging population, chronic periodontitis represents a significant and growing health care burden, despite continuing improvements in dental care. Chronic periodontitis results from complex interactions between an aberrant host response and the plaque biofilm, and evidence is mounting to support the contention that the substantive contribution to tissue damage and bone loss results from an exaggerated host response. Connective tissue alterations arise following host-derived enzyme and oxygen radical release, in response to bacterial toxins and their stimulation of inflammatory mediators. To date, therapeutic strategies have focused on the physical reduction of the microbial challenge by either non-surgical or surgical approaches involving relatively generic, non-specific strategies that ignore the unique inflammatory-immune phenotype of the host. Part of the reason for such an approach relates to our currently limited understanding of the complex mechanisms that underlie the host response.

In some individuals, susceptibility to periodontitis results from altered neutrophilic polymorphonuclear leukocyte (PMN) function or recruitment. One aspect of altered PMN function is that of the production and release of reactive oxygen species (ROS), such as hypochlorous acid (HOCl). HOCl and taurine-N-monochloramine (TauCl) are end-products of the PMN myeloperoxidase-H_2O_2-Cl_2 system. To date, therapeutic use of NaOCl (HOCl sodium salt) and TauCl solutions in periodontitis has not been considered. However, low concentrations of both molecules are associated with compromises in anti-infection defenses, antigen neutralization, and regulation of the inflammatory reaction (Marcinkiewicz *et al.*, 2000; Kontny *et al.*, 2003a; Reeves *et al.*, 2003). Experimental studies suggest that HOCl and TauCl influence redox-regulated cell processes, including modulation of receptors, signaling pathways, and gene transcription (Gopalakrishna and Jaken, 2000; Midwinter *et al.*, 2001; Schieven *et al.*, 2002; Kontny *et al.*, 2003a). Thus, although never previously recognized, HOCl and TauCl may be of potential benefit as adjunctive therapies for periodontitis patients. The objectives of this review are to discuss in detail the possible roles of HOCl and TauCl as novel therapeutic agents, and their likely involvement in the pathogenesis of periodontal diseases.

CHLORINATION AND OXIDATION PROPERTIES OF HOCl AND TAURINE CHLORAMINE

HOCl and TauCl are end-products of the PMN respiratory burst. HOCl results from the myeloperoxidase-catalyzed reduction of hydrogen peroxide by chlorine. HOCl reacts thereafter with its specific intracellular scavenger and powerful reducing agent taurine to yield taurine N-chloramine (TauCl). HOCl and TauCl may also chlorinate amino groups of proteins and amino acids, to produce N-chloramines (Appendix 1). Oxidation reactions are more rapid than chlorination reactions and involve thioether and/or thiol groups of proteins (Peskin and Winterbourn, 2001). The oxidative properties of HOCl (non-specific) and TauCl (specific) explain their capacity to modulate the inflammatory response (Appendix 2).

EFFECTS ON ANTIGENS AND HOST-DERIVED MEDIATORS IN THE EXTRACELLULAR ENVIRONMENT

Direct Antibacterial Activities

Chronic periodontitis results from an enhanced bacterial challenge within periodontal pockets and the release of harmful endotoxins, including lipopolysaccharides (LPS) and gingipains, which may be neutralized by HOCl-induced oxidation and/or chlorination (Kontny *et al.*, 2003a).

Within physiological concentration ranges, HOCl has, *in vitro*, an immediate and highly effective microbicidal activity. HOCl induces irreversible oxidation of various bacterial respiratory electron transporters (Prütz *et al.*, 2001). TauCl mainly generates time-dependent and extended bactericidal properties, which are significantly enhanced within an acidic environment (pH $\approx$ 5) (Marcinkiewicz *et al.*, 2000). Moreover, HOCl and TauCl may repulse some motile bacteria, especially those with flagella and gliding properties; however, the mechanism of this repulsive activity remains unclear (Liu and Fridovich, 1996). HOCl and TauCl-chlorination of proteins, or the proteinaceous part of antigens, increases their immunogeneity, which promotes the presentation of these proteins by antigen-presenting cells (APC), such as monocytes, macrophages, or dendritic cells (Kontny *et al.*, 2003a). HOCl and TauCl-mediated PMN chlorinating activity also plays a role in PMN-macrophage interactions. Chlorination of antigens selectively promotes the non-specific immune response against Gram-negative periodontal pathogens, and reduces the response induced by Gram-positive bacteria. This affects antigen-phagocytosis-activated production of inflammatory mediators by macrophages, but the mechanisms involved remain unclear. Thus, chlorination of endotoxins (such as LPS) released from Gram-negative pathogens does not affect the secretory activity of the macrophage, whereas chlorination of Gram-positive bacteria-released antigens does significantly affect macrophage secretory activity. The release of nitric oxide and Tumor Necrosis Factor-alpha (TNF-α) is decreased, while phagocytosis and Interleukin-6 (IL-6) production are preserved (Marcinkiewicz *et al.*, 1994), but underlying mechanisms are still to be elucidated.

In addition, some harmful exotoxins may undergo oxidative-neutralization. Thus, HOCl-induced oxidation of a crucial cysteine residue of the active site of the gingipains Rgp and Kgp (2 cysteine proteases of *Porphyromonas gingivalis*) may reduce their potentially harmful activity on the periodontal tissues (Curtis *et al.*, 2001).

However, most of these biochemical activities generate, *in vivo*, a loss of HOCl and TauCl antibacterial properties, which results in a spontaneous neutralization of their oxidative activities by the enormous amount of proteins present inside and outside phagocytic vacuoles (Reeves *et al.*, 2003). How effective the oxidation properties of HOCl and TauCl are *in vivo* remains unresolved; nevertheless, such products of the neutrophil respiratory burst induce ideal conditions for microbicidal destruction by proteases rather than their oxidative killing.

Inflammatory Response Modulation

The innate inflammatory response is initiated by a release of histamine from mast cells, leading to a local increase in both capillary pressure and endothelial permeability. Simultaneously, the bacterial LPS both activates acquired immune response and stimulates epithelial cells, fibroblasts, and APCs to produce pro-inflammatory mediators (such as chemokines, IL-1, IL-6, TNFα, GM-CSF, matrix metalloproteinases [MMPs], and prostaglandin PGE_2), that stimulate (Teng, 2003):

(1) recruitment of immune cells (*e.g.*, PMN, monocytes/macrophages, and T-lymphocytes);
(2) connective tissue destruction, due to the production of proteases, MMPs (such as collagenases), and Reactive Oxygen Species (ROS); and
(3) bone resorption that results from Monocyte Chemoattractant Proteins 3 (MCP-3), Macrophage Inflammatory Proteins 1α (MIP-1α), receptor activator of NF-κB ligand (RANK-L), superoxide anions, eicosanoids (PGE_2 and leukotrienes), IL-6, TNFα, and/or IL-1β-mediated osteoclast activation.

HOCl and TauCl possess both pro- and anti-inflammatory properties that may modulate the inflammatory response within the periodontal tissues (Fig. 1). Anti-inflammatory effects appear to predominate and are summarized below:

(1) HOCl-mediated generation of histamine-N-chloramines may modulate histamine activity, tissue distribution, and metabolism within sites of inflammation (Thomas *et al.*, 2000).
(2) TauCl reduces the HOCl-mediated increase in vascular permeability (Tatsumi and Fliss, 1994).
(3) Chemotactic mediators enhance leukocyte adherence to activated endothelium and *in situ* diapedesis.
(4) HOCl and TauCl neutralize various pro-inflammatory cytokines and chemokines (chemotactic factors, leukotrienes, TNF-α, IL-1β, IL-2, and IL-6), regulate metalloproteinases, and release activated growth factors. This activity is related to either a direct oxidation of crucial thiol or thioether residue(s) in these molecules or to an indirect modulating effect on the capacity of α_2-macroglobulins to bind them:
   (a) HOCl inactivates PMN-released leukotrienes, including sulfidopeptidic-LTC4-sulfoxide and 6-trans-LTB4 (Owen *et al.*, 1987), and neutralizes IL-6 (Nishimura *et al.*, 1991).
   (b) In certain conditions, transforming growth factor-β (TGF-β) activation promotes tissue repair and fibrosis. Native TGF-β consists of 2 peptides: an N-terminal one called latency-associated peptide (LAP), and the C-terminal one, called mature TGF-β. In a manner similar to H_2O_2-induced LAP oxidation, HOCl may facilitate access to the active site of the mature TGF-β molecule, resulting in its activation.
   (c) Dysregulation of proteinase activity associated with inflammatory diseases may lead to tissue destruction in periodontitis. HOCl and TauCl seem to play a key role in this regulation through a pathway distinct from that of the tissue inhibitors of matrix metalloproteinases (TIMPs), and appear to reduce the activity of proteolytic enzymes in a concentration-dependent manner (Fu *et al.*, 2003; Reeves *et al.*, 2003). While low concentrations of HOCl activate the proform of matrix

metalloproteinases, collagenase-2, and gelatinase B *via* thiol group oxidation of its cysteine moiety, higher concentrations of HOCl inhibit MMP-7 activation through an oxidative modification of adjacent tryptophan and glycine residues in the catalytic domain (Fu *et al.*, 2003).

(d) Similarly, HOCl inhibits collagenase activities, when the HOCl/collagenase ratio is greater than 40. Moreover, TauCl exerts a direct concentration-dependent inactivation of type VII collagenases, with an IC_{50} = 1.4 mM. HOCl may also inactivate gelatinases when the HOCl/gelatinase ratio is greater than 30, while it does not seem to inhibit them when the ratio is lower than 30 (Michaelis *et al.*, 1992; Davies *et al.*, 1994).

(e) α_2-macroglobulins are plasma molecules that bind and neutralize proteases, cytokines (including TNF-α, IL-1β, IL-2, IL-6, and IL-8), and growth factors including TGF-β, basic fibroblast growth factor (bFGF, also called FGF-2), β-nerve growth factor (β-NGF), and platelet-derived growth factor (PDGF). In plasma, α_2-macroglobulin binding affinity is higher to growth factors (with K_d values in a nanomolar range) than to cytokines (with K_d values in a micromolar range). Consequently, 85-90% of TGF-β and PDGF molecules are inactive, bound to α_2-macroglobulins. HOCl-associated α_2-macroglobulin oxidation induces: (1) a decrease of protease binding, (2) an important increase of α_2-macroglobulin affinity for TNF-α, IL-2, and IL-6 (K_d values in the nanomolar range with a five-fold increase of their binding rate), and (3) a greater decrease of affinity to β-NGF, PDGF-BB, TGF-β1, and TGF-β2 (with a nine- or 13-fold decrease of the binding rate to PDGF-BB and TGF-β2, respectively). In addition, α_2-macroglobulin reacts with methylamine, a nucleophilic primary amine, forming an α_2-macroglobulin-methylamine complex, which can be oxidized by HOCl, leading to a decrease in the binding of various growth factors, without any modification of its affinity to the inflammatory cytokines (Wu *et al.*, 1998).

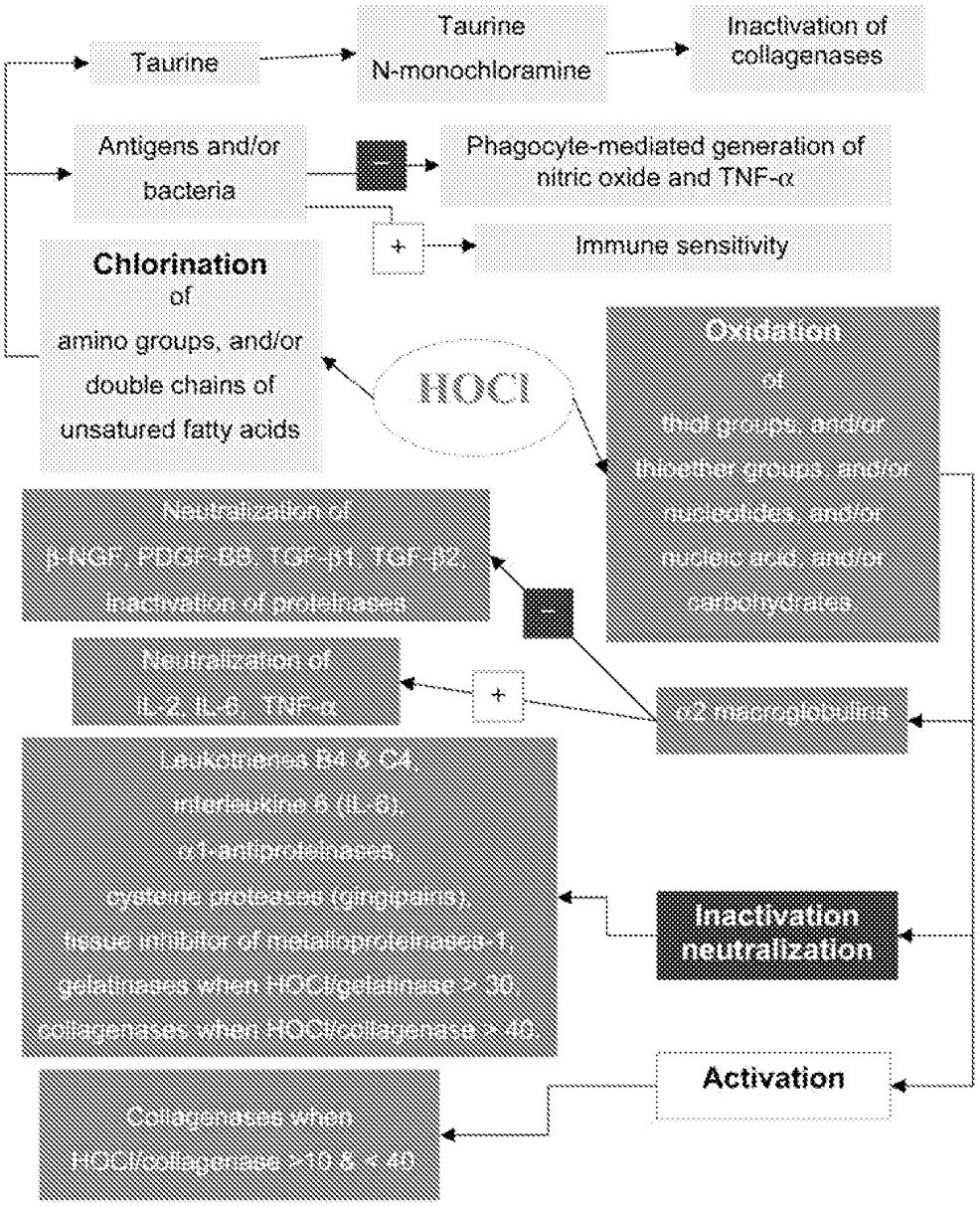

Figure 1. Extracellular activities of HOCl and TauCl. IL, interleukin; β-NGF, β-nerve growth factor; PDGF-BB, platelet-derived growth factor-BB; TGF-β1, transforming growth factor β1; TGF-β2, transforming growth factor β2; TNF-α, tumor necrosis factor α.

By contrast, HOCl and TauCl may, in certain conditions, exert a deleterious stimulation of inflammatory processes. In the defense reaction against bacteria, numerous enzymes are released by leukocytes into the extracellular environment, a mechanism that appears to be essential in periodontitis. Metalloproteinases—including sulfur, magnesium, iron, zinc, or calcium-dependent endopeptidases, such as collagenases—are involved in such tissue damage.

Table. *In vitro* Inhibition of Inflammatory Mediators and Reactive Oxygen Species (ROS) Production with HOCl or TauCl: Inhibitory Concentrations and the Experimental Models Reported in the Literature

Inflammatory Mediators & Reactive Oxygen Species (ROS)[a]	Taurine N-monochloramine (TauCl) Inhibitory Concentrations	Hypochlorous Acid (HOCl) Inhibitory Concentrations	Experimental Model *in vitro*	References
Chemokines MCP-1 & -2	IC_{50} ~ 500 μM	/	LPS & INF-γ-activated rat alveolar macrophages	Liu *et al.*, 2003
IL-1β	400 μM	/	LPS-activated human adherent monocytes	Park *et al.*, 2002
	IC_{50} ~ 250 μM		Secreted IL-1β by LPS-activated human PBMC[b]	Chorazy *et al.*, 2002
	IC_{50} ~ 300 μM		Cell-associated IL-1β by LPS-activated human PBMC	Chorazy *et al.*, 2002
IL-2	400 μM	/	Non-adherent human leukocytes	Park *et al.*, 2002
	IC_{50} ~ 250 μM	/	Mouse T-lymphocyte response to various antigens and mitogens	Kontny *et al.*, 2003a
IL-6	IC_{50} ~ 200 μM	/	LPS & INF-γ-activated murine dendritic cells	Marcinkiewicz *et al.*, 2000
	400 μM	/	Adherent monocytes & non-adherent human leukocytes	Park *et al.*, 2002
	IC_{50} ~ 250 μM	/	Mouse T-lymphocyte response to various antigens and mitogens	Kontny *et al.*, 2003a
	IC_{50} ~ 250 μM	/	LPS & INF-γ-activated mouse PMN	Kontny *et al.*, 2003a
	IC_{50} ~ 200 μM	/	LPS-activated human PMN	Park *et al.*, 2002
	IC_{50} ~ 225 μM	/	IL-1β-activated human fibroblast-like synoviocytes	Kontny *et al.*, 2003a
	IC_{50} ~ 425 μM	/	Secreted IL-6 by LPS-activated human PBMC	Chorazy *et al.*, 2002
	IC_{50} ~ 400 μM	/	Cell-associated IL-6 by LPS-activated human PBMC	Chorazy *et al.*, 2002
IL-8	400 μM	/	Adherent monocytes & non-adherent human leukocytes	Park *et al.*, 2002
	IC_{50} ~ 400 μM	/	LPS-activated human PMN	Park *et al.*, 2002
	IC_{50} ~ 450 μM	/	IL-1β-activated human fibroblast-like synoviocytes	Kontny *et al.*, 2003a
TNF-α	IC_{50} ~ 500 μM	IC_{50} < 100 μM	LPS & INF-γ-activated mouse macrophages	Marcinkiewicz *et al.*, 2000
	IC_{50} ~ 250 μM	/	LPS & INF-γ-activated mouse PMN	Kontny *et al.*, 2003a
	IC_{50} ~ 460 μM	/	Secreted TNF-α by LPS-activated human PBMC	Chorazy *et al.*, 2002
	IC_{50} ~ 480 μM	/	Cell-associated TNF-α by LPS-activated human PBMC	Chorazy *et al.*, 2002
PGE_2 (COX-2 proteins)	IC_{50} ~ 250 μM	/	LPS & INF-γ-activated murine dendritic cells	Kontny *et al.*, 2003a
	IC_{50} ~ 400 μM	/	LPS & INF-γ-activated murine macrophages	Quinn *et al.*, 2003
	IC_{50} ~ 250 μM	/	LPS & INF-γ-activated mouse PMN	Kontny *et al.*, 2003a
	IC_{50} ~ 300 μM	/	IL-1β-activated human fibroblast-like synoviocytes	Kontny *et al.*, 2003b
PGE_2 (COX-2 mRNA)	IC_{50} ~ 400 μM	/	IL-1β-activated human fibroblast-like synoviocytes	Kontny *et al.*, 2003b
Nitric Oxide (NO) (iNOS gene)	IC_{50} ~ 250 μM	/	LPS & INF-γ-activated murine dendritic cells	Kontny *et al.*, 2003a
	IC_{50} ~ 250 μM	IC_{50} ~ 80 μM	LPS & INF-γ-activated mouse macrophages	Marcinkiewicz *et al.*, 2000
	IC_{50} ~ 250 μM	/	LPS & INF-γ-activated mouse PMN	Kontny *et al.*, 2003a
LCL[c]	IC_{50} ~ 550 μM	IC_{50} ~ 100 μM	Peritoneal mouse neutrophil PMN	Marcinkiewicz *et al.*, 2000
Hydrogen peroxide (H_2O_2)	/	IC_{50} ~ 80 μM	Peritoneal mouse neutrophil PMN	Marcinkiewicz *et al.*, 2000
Myeloperoxidase	/	IC_{50} < 100 μM	Peritoneal mouse neutrophil PMN	Marcinkiewicz *et al.*, 2000
Superoxide anion (O_2^-)[d]	IC_{50} ~ 100 μM	/	PMA-activated human PMN	Park *et al.*, 1998

[a] HOCl is able to generate the same ROS inhibition as TauCl, but requires dramatic cell anti-oxidant depletion (Prütz *et al.*, 2001).
[b] PBMC: peripheral blood mononuclear cells.
[c] Luminol-dependent chemiluminescence (LCL) is commonly attributed to ROS production during the neutrophil respiratory burst. The difference between TauCl and HOCl inhibitory titers may be explained by their differential cell uptake (Kim *et al.* 1998) and/or their biochemical reactivity and specificity.
[d] TauCl-induced inhibition of the superoxide anion is dose-dependent and related to NADPH oxidative inactivation.

(1) As mentioned above, low concentrations of HOCl may activate the proform of matrix metalloproteinases, gelatinase B, and collagenases (which hydrolyze I, II, III native collagens). Collagenase stimulation is observed only when the HOCl/collagenase ratio is lower than 40.
(2) HOCl may also inhibit α_2-macroglobulin-related neutralization of cell proteases, whereas both HOCl and TauCl inactivate the α_1-proteinase inhibitor (Evans and Pryor, 1994; Wu *et al.*, 1998).
(3) HOCl may interfere with the c5 component of the complement cascade, which, on activation, generates 2 fragments, the c5b fragment with antibacterial membrane-lytic activity, and the c5a fragment with PMN chemotactic properties. HOCl- and TauCl-induced oxidation of methionine residues in the c5 fragment generates structural changes that result in its activation (Vogt, 1996).
(4) HOCl promotes macrophage adherence to endothelium and enhances endothelium permeability (Tatsumi and Fliss, 1994).
(5) HOCl and TauCl promote the innate immune response against Gram-negative bacteria (unlike Gram-positive species), *via* chlorination of antigens (Marcinkiewicz *et al.*, 1994).

In summary, both HOCl and TauCl modulate the inflammatory response, often in a concentration-dependent manner. The anti-inflammatory effects would appear to predominate, but the outcome of these multiple affects *in vivo* requires further exploration. To date, no clinical trials have investigated the effects of a HOCl and TauCl combination in human or animal periodontal therapy.

EFFECTS ON MEDIATOR PRODUCTION AND INTRACELLULAR SIGNAL TRANSDUCTION PATHWAYS

Inflammatory Mediator and Enzyme Production

In the course of periodontitis, pathogens and their products induce (i) an activation of monocytes/macrophages and CD4+ T-helper type-1 (Th1) cells, while (ii) Th2 cell activity may be neutralized. This generates (i) a stimulation of IL-1β, TNF-α, IL-6, IL-2, and INF-γ production, and (ii) a defective synthesis of IL-4 and IL-10 (Górska *et al.*, 2003). IL-1 and TNF-α release results in an intensive recruitment of inflammatory cells with: (1) chemokine production, including MCP-1 and the MIP family; (2) activation of endothelial cell leukocyte adhesion molecules; and (3) stimulation of both adherent and non-adherent leukocytes. The result is a stimulation of PMN degranulation, ROS production, and MMP synthesis. Moreover, PGE_2 production is activated. All these inflammatory molecules generate potentially harmful activity, resulting in connective tissue damage, alveolar bone resorption, and periodontal clinical attachment loss (Górska *et al.*, 2003; Teng, 2003). Consequently, neutralization of this inflammatory cascade appears crucial to periodontal healing.

TauCl and HOCl inhibit *in vitro* the cell production of various inflammatory mediators and ROS (Table). TauCl significantly reduces the production of IL-1β, IL-6, and IL-8 in LPS-stimulated human adherent monocytes, and also inhibits lymphocyte proliferation (Park *et al.*, 2002). In LPS-stimulated murine peritoneal macrophages, TauCl may interfere with the transduction signals which generate MMP-9 expression (Park *et al.*, 2000). TauCl-related inhibition of MCP-1, MIP-2, IL-1β, IL-2, IL-6, IL-8, TNF-α, nitric oxide (NO), and PGE_2 production involves the nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1) transcriptional pathways (Fig. 2), leading to partial or complete inhibition (Kontny *et al.*, 2003a; Liu *et al.*, 2003).

Regulation of Redox-sensitive Transcription Factors

NF-κB and AP-1 are redox-sensitive transcription factors, whose control has been proposed as a potentially important host-modulation strategy in periodontitis (Chapple *et al.*, 2002). The NF-κB proteins are comprised of homo- and hetero-dimers belonging to the Rel protein family. They are responsible for the transcription of many genes, including those regulating inflammation, acquired immunity, cell-to-cell interactions, cell apoptosis, and proliferation. The NF-κB dimer is associated with an IκB inhibitory protein, which masks its nuclear location signal site and maintains NF-κB in its latent form within the cytoplasm. AP-1 is a two-gene-dependent transcription factor (Jun and Fos). The monomers (c-Jun, c-Fos, v-Jun, v-Fos, Fos, Fra-1, Fra-2, Jun-D, Jun-B, and ATF) can generate a homodimeric complex (Jun/Jun) or a heterodimeric complex (Jun/Fos). This transcription factor family is critical to the early genetic regulation of immune responses.

NF-κB and AP-1 are stimulated by a specific mitogen-activated protein kinase (MAP-kinase) pathway. Cell receptors contain an extracellular binding site, a transmembrane domain, and a cytoplasmic domain, which exerts a catalytic kinase activity. Receptor-linked protein-tyrosine kinase activation induces a cascade of transduction signals, involving MAP-kinases. Briefly, the tyrosine residue phosphorylation of the membrane receptor indirectly induces the recruitment and activation of MAP-kinase-kinase kinases (MAPKK-kinases), mainly through tumor-necrosis-factor receptor-associated factor (TRAF) proteins. MAPKK-kinases are serine-threonine kinases. Their activation leads to phosphorylation and activation of MAPK-kinases, which in turn may phosphorylate critical threonine and tyrosine residues in MAP-kinases. MAP-kinases have the potential to phosphorylate other cytoplasmic proteins, which may activate transcription factors (like AP-1, NF-κB, Stat3, Gadd153/CHOP, and the Smad family), inducing their translocation from the cytoplasm to the nucleus (Gopalakrishna and Jaken, 2000).

NF-κB and AP-1 are activated by specific MAP-kinases, called, respectively, IκB kinases (IKK) and c-Jun N-terminal kinase (JNK). Two IKK homologues can be distinguished: IKK-α and IKK-β. This inducible serine phosphorylation leads to a polyubiquitination of adjacent lysines, followed by a 26S proteasome-dependent degradation of IκB, thus releasing NF-κB, which translocates to the nucleus and binds to DNA. Similarly, for example, JNK phosphorylates c-Jun, following TNF-α-receptor stimulation, thereby inducing AP-1 activation (Appendix 3).

NF-κB is known to regulate the production of numerous inflammatory mediators (such as IL-1α, IL-1β, IL-2, IL-6, TNF-α, NO, PGE_2, TGF-β, and adhesion molecules) and an inhibitor of apoptosis proteins (IAP), whereas AP-1 regulates the production of some cytokines (*e.g.*, IL-8) and MMPs. All these mediators and proteins are involved in periodontal diseases, and their physiological inhibition seems to be crucial

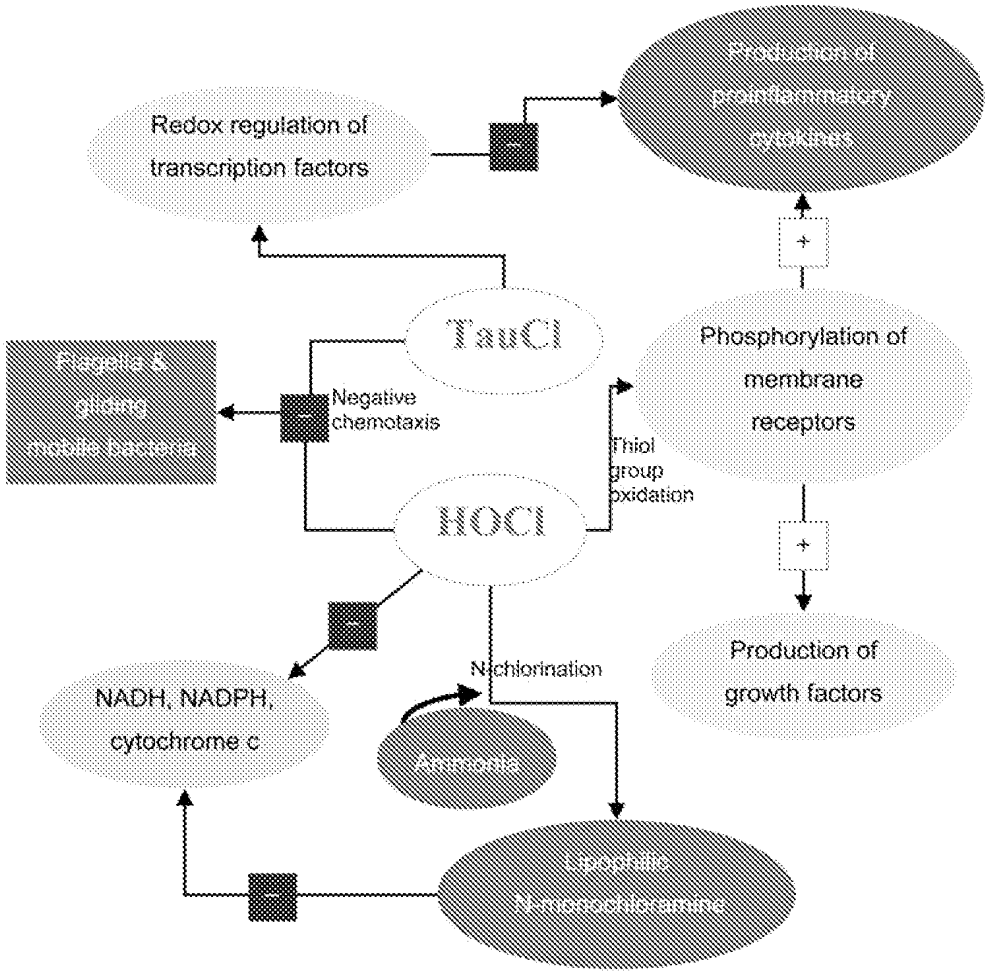

Figure 2. Intracellular activities of HOCl and TauCl.

to periodontal tissue turn-over, and to triggering the processes of regeneration (Górska *et al.*, 2003; Teng, 2003). TauCl inhibited the NF-κB-related transcription of inducible nitric oxide synthase (iNOS) and TNF-α genes in a rat model of broncho-alveolar macrophages (Barua *et al.*, 2001), and iNOS, cyclo-oxygenase-2 (COX2), TNF-α, MCP-1, and MIP-2 genes in rat cortical astrocytes (Liu *et al.*, 2003). TauCl reduced both the translocation and NF-κB DNA-binding activities, and maintained cytoplasmic levels of unphosphorylated IκB-α, probably as a complex with NF-κB (Quinn *et al.*, 2003). The TauCl-induced inhibition of IκB kinase (IKK) activity is suspected on an upstream key kinase or thioredoxin-dependent redox protein. By contrast, in human Jurkat T-cells, the TNF-α-stimulated luciferase gene expression is NF-κB-controlled. TauCl reduces IKK activation at a downstream rather than an upstream level, in the kinase cascade. This TauCl-generated inhibition does not occur on serine 32/36 phosphorylation, but results from an oxidation of IκB-α methionine 45, yielding a sulfoxide residue. This oxidation is likely to induce a spatial structure change that masks serine 32/36, preventing phosphorylation, or avoids phosphorylated IκB-α recognition by F-box protein and subsequent lysine 21/22 ubiquitination (Kanayama *et al.*, 2002).

The level of TauCl-inhibition for each above-mentioned mediator depends on NF-κB involvement in its production. Furthermore, these data do not hide the existence of other TauCl-mediated inhibitory mechanisms. Thus, in HFLS, the inhibition of COX2 gene NF-κB-transcription (IC_{50} ~ 400 μM) is less sensitive than post-transcriptional events (IC_{50} ~ 300 μM), suggesting that the main inhibition occurs at the post-transcriptional level (Kontny *et al.*, 2003b). In the same way, TauCl essentially suppresses the translation of TNF-α mRNA (Park *et al.*, 2002). Therefore, TauCl has the ability to inhibit the production of the principal inflammatory mediators involved in the pathogenesis of periodontitis. These inhibitions may involve activity at the level of gene transcription, at the post-transcriptional stage, and/or at mRNA translation. Consequently, TauCl not only protects tissues against excess HOCl (an anti-oxidant effect), but also possesses anti-inflammatory properties.

Tissue-regenerative Activity

Cessation of tissue destruction, both by direct neutralization and by cell inhibition of pro-inflammatory mediators, could promote healing (Appendix 4). The induction of periodontal tissue regeneration is known to require an adequate production of cellular growth factors, such as insulin-like growth factor (IGF), epidermal growth factor (EGF), keratinocyte growth factor (KGF, also called FGF-7), FGF-1 and -2, TGF-β, PDGF, vascular endothelium growth factor (VEGF), connective tissue growth factor (CTGF), and/or cementum-derived growth factor (CGF) (Grzesik and Narayanan, 2002). Protein tyrosine kinases and protein tyrosine phosphatases (PTPs) form complexes involving membrane receptors for certain growth factors, and receptors that regulate the synthesis of many inflammatory and regenerative molecules, including IGF-1, bFGF (FGF-2), EGF, NGF, and/or PDGF. In these complexes, PTPs negatively control phosphotyrosine protein kinases through a tyrosine phosphorylation inhibition. PTP activation involves the reduction of one specific thiol residue within the active site. All PTPs share a CXXXXXR active site motif (where C is a cysteine, R an arginine, and X any amino acid). In the low-molecular-weight phosphotyrosine-protein phosphatases (LMW-PTP), present in many mammalian tissues, cysteine-12 and cysteine-17 are conserved residues in the catalytic site. Oxidative stress induces the production of intracellular H_2O_2. H_2O_2-mediated oxidation inhibits LMW-PTP by producing a disulfide bond between cysteine-12 and cysteine-17 (Chiarugi *et al.*, 2001). Similarly to H_2O_2, an oxidative action of other non-specific lipophilic oxidants, such as HOCl or NH_2Cl, on these strategic thiol residues may be hypothesized (Fig. 2). To date,

there is no definitive demonstration of a HOCl-induced direct activation of receptor-tyrosine-kinases, although recent experimental results suggest its existence. In human B- and T-lymphocytes, HOCl induces (similarly to H_2O_2) a protein tyrosine phosphorylation and activates the zeta-associated protein-70 (ZAP-70) tyrosine kinase, generating a tyrosine-kinase-dependent calcium signaling. Furthermore, HOCl may bypass cell membrane receptors to stimulate intracellular protein tyrosine kinases and calcium signaling directly. In this case, cell receptor activation may prolong the HOCl-generated calcium signal. Activation of these directs intracellular transduction pathways and stimulates TNF-α gene transcription in human peripheral blood mononuclear cells (Schieven *et al.*, 2002). HOCl-mediated effects on calcium signaling pathways may, in turn, induce intracellular modifications, including HOCl calcium-dependent production (Blackburn and Chatham, 1994).

As mentioned above, the production of pro-inflammatory cytokines, growth factors, and extracellular matrix components requires the MAP-kinase-mediated activation of transcription factors, such as AP-1, NF-κB, Stat3, Gadd153/CHOP, and the Smad family. Moreover, most of these factors bind to their specific membrane receptors and activate signal transduction pathways, which may involve MAP kinase activation (such as VEGF or PDGF). In contrast to H_2O_2, HOCl does not significantly activate JNK, except at a lethal dose (50 μM). The extremely low doses of HOCl required for MAP kinase activation (20-50 μM), compared with H_2O_2 (400-1000 μM), may result from the high reactivity of HOCl with thiol groups and not physiological enzymatic degradation. HOCl-induced MAP kinase activation may be due to a direct thiol residue substitution into sulfenic/disulfide and/or an intracellular redox-status change. These hypotheses require further investigation (Midwinter *et al.*, 2001). Protein-kinase C may also activate MAP kinases. It possesses cysteine-rich regulation and catalytic domains. More precisely, the regulation domain possesses 4 crucial zinc fingers that allow for binding to membrane lipids. Each zinc finger contains 6 regulatory spaced cysteine residues that may generate a fold structure, leading to coordination between 2 zinc atoms. The zinc-thiolate structure is positively charged and is highly susceptible to negatively charged lipophilic oxidants, like hypochlorite. These oxidants induce the loss of zinc finger conformation, thereby activating protein-kinases-C through calcium- and phospholipid-independent pathways (Gopalakrishna and Jaken, 2000). However, since protein-kinases C are partially located inside the cell membrane, other negatively charged but hydrophilic agents, such as TauCl, are not active. Although not yet described, a synergistic effect between protein-kinase-C phosphorylation and oxidation has been suggested. Interestingly, the zinc finger structure is a crucial part of various other signaling proteins, including TRAF proteins, c-myc, Raf, some DNA-repair enzymes, transcription factors of the steroid-binding superfamily, and the transcription factor protein 53 (p53). This may explain their potential sensitivity to negatively charged oxidants.

HOCl is a non-specific lipophilic oxidant, has a rapid rate of cell-uptake, and preferentially oxidizes thiol residues. This redox activity is more powerful than H_2O_2. At toxic concentrations, HOCl generates a rapid and irreversible loss of intracellular protein-thiol groups, including glutathione, glutaredoxin, and thioredoxin, through an irreversible oxidative cross-linking and aggregation process (Pullar *et al.*, 2001). Low HOCl levels oxidize preferentially accessible thiol residues and, more specifically, cysteine residues of vital cellular anti-oxidants, such as reduced glutathione, thioredoxin, and glutaredoxin. Thioredoxin expression modulates NF-κB activity at 3 levels. In the nucleus, it helps NF-κB to bind to DNA (Harper *et al.*, 2001). In the cytosol, it activates NF-κB at a downstream level of NIK (Hirota *et al.*, 2000), while, near the cell membrane, it inhibits NF-κB-mediated cytokine production, at an upstream level of NIK and at a downstream level of TRAFs (Takeuchi *et al.*, 2000). In contrast, glutaredoxin expression increases NF-κB activation and, as well as thioredoxin, increases AP-1 activation. HOCl may oxidize cytoplasmic anti-oxidants close to the cell membrane, and thus indirectly regulates these transcription factors. Moreover, non-specific oxidants, like H_2O_2 and HOCl, may modulate IκB kinase activities, with the generation of an inducible phosphorylation on the tyrosine 42 residue instead of the serine 32/36 residues of IκB-α, resulting in an IκB-α dissociation from NF-κB, without its degradation by the 26S proteasome (Janssen-Heininger *et al.*, 2000).

Thus, although HOCl has the ability to inhibit the redox-sensitive transcription factors, similarly to TauCl, anti-oxidant-mediated HOCl neutralization prevents this activity *in vivo*. In fact, an HOCl-induced moderate depletion of anti-oxidants may favor the HOCl-mediated non-specific activation of protein tyrosine kinases, MAP kinases, and/or protein kinases C, which leads to non-specific pro-inflammatory gene transcription. Therefore, non-toxic HOCl concentrations induce cell proliferation and stimulate extracellular matrix component production in human fibroblasts (Hidalgo and Dominguez, 2000).

CONCLUSION

In addition to their anti-infectious properties, the end-products of the PMN respiratory-burst, HOCl and TauCl, possess, at low concentrations, very interesting and complementary activities which modulate crucial phases of the inflammatory process, cell turnover and periodontal tissue healing. Such activities may be summarized as follows: (1) neutralization of antigens and pathogenic agents following chlorination and/or their oxidation; (2) direct neutralization of metalloproteinases, IL-5 and IL-6; (3) enhancement of inflammatory mediator binding to α_2-macroglobulins; and (4) reduction of the binding of growth factors to α_2-macroglobulins. However, differences exist between HOCl and TauCl activities. Extracellular HOCl stimulates membrane receptors and activates kinase cascades, leading to the production of native compounds of the extracellular matrix and/or cytokines (including growth factors and pro-inflammatory cytokines), whereas TauCl inhibits most of the production of pro-inflammatory cytokines and reactive oxygen species. The hydrogen peroxide/myeloperoxidase system generates HOCl and TauCl as end-products. Thus, a deficient production of both HOCl and TauCl may play a key role in the pathogenesis of periodontal diseases. Further investigation into the dynamic interactions of these 2 molecules—not only with each other, but also with components of the periodontal connective tissues and inflammatory-immune system—may lead to new opportunities for periodontal therapy, based upon the modulation of the host response in susceptible patients.

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An overview of nonsurgical and surgical therapy

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Periodontal treatment traditionally comprises initial nonsurgical debridement followed by a reevaluation, at which stage the need for further treatment, usually surgical in nature, is established (Fig. 1). This paper will provide an overview of available evidence pertaining to the efficacy of these phases in the treatment of periodontitis patients.

Nonsurgical therapy

Conventional nonsurgical periodontal therapy consists of mechanical supra- and subgingival tooth debridement and instruction in self-administered oral health care measures. These measures are directed towards reducing the bacterial load and altering the microbial composition towards a flora more associated with health. In turn, these microbiologic changes result in lower levels of inflammation and relative stability in periodontal attachment levels (62, 75).

Studies have been performed to demonstrate whether complete removal of all subgingival calculus was possible, whether there were differences in calculus removal between hand and ultrasonic instruments and whether differences in calculus removal relate to operator experience and different teeth and sites. Hopeless teeth scheduled for extraction for periodontal reasons were subjected to subgingival debridement prior to their removal. After extraction, subgingival areas were examined microscopically for residual calculus under ×10 magnification. The results suggest that complete removal of subgingival calculus does not seem to be predictably attainable following subgingival instrumentation (23). Small areas of calculus are often left behind, with anywhere from about 3% to 80% of instrumented root surfaces showing some residual calculus (10, 23, 72, 76). It was also observed that more calculus is left behind on proximal surfaces, in deep sites, and in furcation areas (11, 14, 23, 55, 78). Furthermore, it was pointed out that a similar degree of calculus removal can be accomplished with hand and ultrasonic instruments (23, 32, 42, 89). In addition, operator experience has been shown to be an important factor in the effectiveness of calculus removal (9, 23, 24, 42). Hand instrumentation, ultrasonic, and sonic instrumentation seem to lead to similar clinical improvements in patients with advanced periodontitis (2, 51).

Periodontal instrumentation is aimed at effectively removing plaque and calculus without excessively

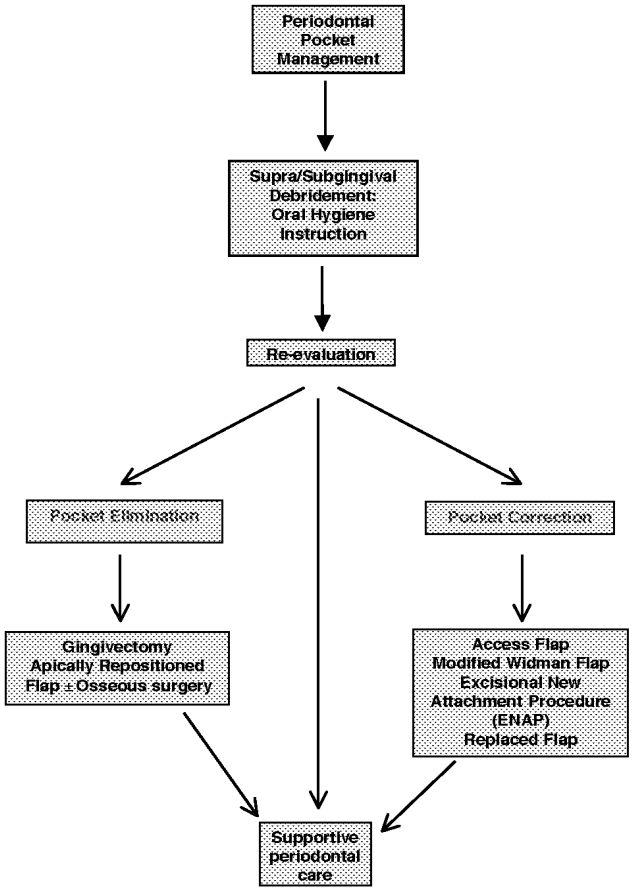

Fig. 1. Schematic representation of typical treatment modalities and their sequence of use in periodontal pocket management.

instrumenting the tooth surfaces. Overinstrumentation can lead to hypersensitivity and pulpitis due to excessive cementum and dentin removal (25, 28). Extensive instrumentation may also cause various degrees of increased surface roughness in both supra- and subgingivally located areas, which in turn may enhance plaque retention (39, 45, 73, 82, 85). Results of studies investigating the degree of roughness following the use of hand and sonic/ultrasonic instruments are difficult to interpret because critical variables such as forces applied during instrumentation were often not reported (90). Nevertheless the finding that different instruments lead to the same clinical results seems to suggest that variations in root surface roughness do not affect overall healing (23).

In the past, endotoxin or lipopolysaccharide derived from cells of gram-negative bacteria was thought to be so firmly attached to the root surface that extensive cementum removal was required during subgingival instrumentation (1). More recent studies on extracted teeth indicate that endotoxins are superficially bound and can be removed by such means as brushing. Thus systematic root planing to remove cementum does not seem warranted (15, 29, 77).

Furcation opening is often less than 1 mm, too small to be effectively reached with relatively larger curettes (33). Most of the new ultrasonic tips are approximately 0.50 mm in diameter, which may favor ultrasonics as the instruments of choice for furcation sites. One study on instrumentation of furcations with and without surgical access indicates that no major differences were observed between use of curettes or ultrasonics in the closed treatment groups and in wide furcations. Ultrasonics performed better for narrow furcation areas and in the open treatment groups (55). On the basis of the above and other studies, power-driven instruments may be considered more efficient for calculus removal in general and in furcation sites (21, 38, 41, 44, 50, 52, 79, 86).

Effectiveness of nonsurgical therapy

Nonsurgical therapy for the control of periodontitis normally consists of subgingival debridement combined with oral hygiene instruction. Subgingival debridement in the absence of adequate oral hygiene measures results in a limited healing response (53). Other studies employing control groups whose treatment was confined to oral hygiene alone corroborate these results (23). Oral hygiene instruction in the absence of subgingival debridement also results in a suboptimal clinical response (13, 51, 84).

Badersten et al. (2) studied the amount of improvement that results for nonmolar sites from the combined effects of oral hygiene and supra- and subgingival debridement in patients with advanced periodontal disease. Mean plaque scores were reduced to <20% and mean bleeding scores to <20% irrespective of the initial pocket depth. Probing depths for initially deep sites were reduced by a combination of gingival recession and gain of probing attachment level (for sites with initial probing depth around 8 mm, the depth was reduced to an average of about 5 mm due to 2 mm of gingival recession and 1 mm of improved gingival adaptation at the base of the lesion).

These mean results were maintained over 2 years of observation. Furthermore, the magnitude of the pocket depth appeared to have no effect on the efficacy of the therapy in maintaining clinical attachment levels.

Analysis of outcomes using mean results of pooled sites within a patient can mask deterioration in a subset of sites under study. Identification of such sites is complicated for the following reasons:

- Probing depths and probing attachment levels (measurements made from a fixed point on the tooth, e.g. cementoenamel junction) are subject to reproducibility error. This has resulted in various strategies to avoid sites being labeled in error as deteriorating sites, for example the use of thresholds of change large enough to overcome the error, or the use of means of multiple recordings at any one-time point.
- Probing measurements do not disclose the true connective tissue levels attached to teeth. In inflamed states the probe penetrates the base of the junctional epithelium, whereas in noninflamed states the probe stops short of the base of the junctional epithelium (12, 26, 49, 80, 81). Thus, probe tip position and therefore probing depth could vary at different times due to tissue inflammatory status without any change in connective tissue levels. Such variation is reportedly in the region of 1–1.5 mm (26). This leads to a possible validity error in designating sites as having ongoing disease.

Failure to compensate for these potential sources of error may result in sites being erroneously diagnosed as having undergone connective tissue loss. Analyses carried out on the data of Badersten et al. (2) used linear regression analysis (to compensate for reproducibility error) and a threshold of 1.5 mm change (to compensate for validity error) to identify sites that showed progression over an observation period of 2 years (23). Forty-nine subjects were included and only nonmolar sites were studied. Overall, 120 sites

(5%) showed ongoing loss. Of interest were the characteristics of these sites; 14% were initially ⩾ 7.0 mm deep and 48% were ⩽ 3.5 mm deep; 44% were either buccal or lingual sites that were often shallow and therefore not those sites that clinicians would expect to show progression. The question remained as to the nature of the attachment loss in shallow sites following initial therapy. When effects on probing attachment levels due to instrumentation trauma were accounted for and furcation sites included, 4% of sites exhibited ongoing attachment loss and most of these had probing depths and other inflammatory characteristics not suggestive of disease but of a remodeling process (16, 19).

These results for frequencies of sites with ongoing loss are, however, not representative of deep molar furcation sites. Nordland et al. (59) found a lower mean response for probing depth and probing attachment change and nearly twice the frequency of ongoing loss in deep molar furcation sites as other comparable molar sites or nonmolar sites (Fig. 2 and 3). Loos et al. (52) reported similar results for furcation sites with 12% of sites ⩽ 3.5 mm, 11% of sites 4–6.5 mm and 27% of sites ⩾ 7 mm deteriorating over a 2-year observation period. This unfavorable healing response has also been found in other studies (23).

The presence of root furrows in nonmolar teeth may also compromise healing. Apart from these anatomic aspects, tooth types and location in the dentition seem to have little impact on treatment outcome (23). Experienced operators can obtain an adequate debridement in one episode of instrumentation with either hand or sonic / ultrasonic instruments (2, 3, 50).

Comparison of attachment levels before and immediately after subgingival instrumentation have found an average probing attachment loss of about 0.5 mm, irrespective of initial probing depth, the histologic nature of which has not been fully clarified. The loss may be explained either by a lateral displacement of the gingival tissues or by tearing of coronal connective tissue fibers, facilitating apical probe penetration immediately after treatment. It has been suggested from animal experiments that if connective tissue damage has been a result of instrumentation, this may be irreversible and healing occurs by apical migration of junctional epithelium (48). Probing measurements conducted a few months after treatment do not disclose the initial traumatic probing attachment loss in the majority of sites, perhaps because of subsequent readaptation of the gingival tissues (16, 19).

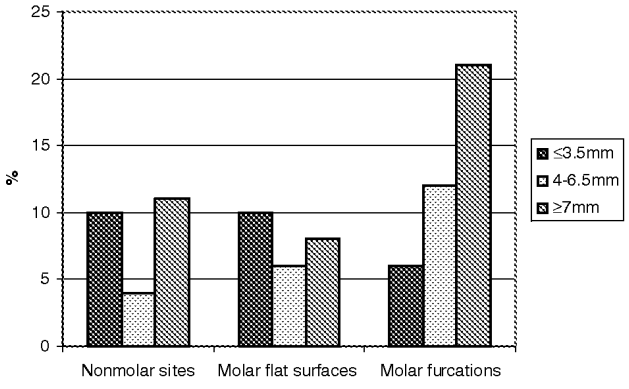

Fig. 2. Percentage of sites with probing attachment loss at 0–24 months by initial probing depth (from Nordland et al. (59)).

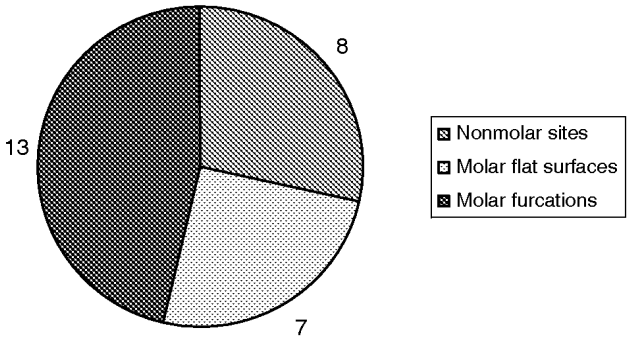

Fig. 3. Percentages of losing sites at 0–24 months by surface location (from Nordland et al. (59)).

The clinical characteristics of sites with ongoing attachment after initial periodontal treatment are remarkably variable (17). Sites with probing attachment loss following nonsurgical treatment may or may not display the clinical characteristics commonly associated with periodontitis (deepened probing depth, increased bleeding tendency, and possibly suppuration). A classification into 'questionable' periodontitis was employed (deterioration that may not be associated with chronic inflammatory signs). It was found that 21–35% of all sites with attachment loss fell into this category. The authors speculated as to the cause of probing attachment loss for the 'questionable periodontitis' sites. The following conjectural reasons were suggested:

- trauma from instrumentation during initial treatment and during maintenance treatment.
- trauma from toothbrushing and other home care procedures.
- remodeling of the marginal periodontal tissues as an effect of the improved and changed conditions after treatment;

- gradual recession of the periodontal tissues related to the aging process.
- remodelling of the periodontal structures associated with a process of continuous eruption of the teeth.

It is also possible that a form of periodontitis of microbial origin can occur in the absence of pronounced clinical signs of disease.

Bollen et al. (7), Mongardini et al. (56), Quirynen et al. (66), and recently De Soete et al. (22) studied the hypothesis that plaque control and root debridement might be enhanced by a concomitant 'full-mouth disinfection'. This initially involved scaling and root planing of all quadrants within 24 h in combination with the application of chlorhexidine to all intraoral niches for 2 months both in the dental surgery and at home. Compared to a conventional, quadrant-by-quadrant approach to nonsurgical treatment, clinical and microbiologic parameters showed improved results following periodontal debridement completed within 24 h combined with simultaneous and postoperative full-mouth disinfection. These results confirm those of a similar study by Bollen et al. (8). The findings suggest that re-infection of treated sites during the healing phase may occur from remaining untreated sites, or from other niches in the oral cavity. Quirynen et al. (65), studied the relative importance of the use of chlorhexidine in the full-mouth disinfection. Clinical and microbiologic results from the studies indicated that chlorhexidine had no adjunctive effects. However, its use may be advisable in patients with a low compliance and because it aids initial healing.

Re-evaluation

Re-evaluation of results following initial treatment is critical for adequate selection of additional therapy and for establishing the best possible long-term prognosis. Traditionally, re-evaluation is performed a few months after initial periodontal treatment. Although data demonstrate that healing may continue for a period of 9 months following initial therapy, most of the healing seems to be complete at 3 months following therapy (2). Records of dental plaque, bleeding on probing, suppuration on probing, and probing depth obtained at four to six sites around each tooth are compared to records taken before baseline. Tables 1 and 2 present estimates, generally observed in studies, of mean changes from baseline to re-evaluation in clinical parameters.

Table 1. Estimations generally observed in studies of improvements in plaque and bleeding scores for sites of different initial probing depths after a single episode of supra- and subgingival instrumentation

Initial probing depth	Plaque	Gingival bleeding
⩽ 3.5 mm	≈ 50% → 10%	≈ 55% → 15%
4–6.5 mm	≈ 80% → 15%	≈ 80% → 25%
⩾ 7 mm	≈ 90% → 25%	≈ 90% → 30%

Table 2. Mean changes, generally observed in studies in probing depth, probing attachment levels, and gingival recession after a single episode of supra- and subgingival instrumentation

Initial probing depth	Probing depth	Probing attachment level	Gingival recession
⩽ 3.5 mm	0	−0.5	0.5
4–6.5 mm	1–2	0–1	0–1
⩾ 7 mm	2–3	1–2	1–2

The measurement of true periodontal pockets has traditionally been used in the diagnosis of periodontal disease. Longitudinal changes in probing depth may result from alteration in the gingival margin level and/or from influences at the pocket base. In recent times it has been thought more appropriate to focus on events at the base of the pocket as a measure of response to treatment. Probing attachment levels, recorded longitudinally from a fixed point on the tooth are used for this purpose and are normally used as a gold standard, although their validity when used in isolation from other parameters has been challenged (16).

By convention, deep probing depths and inflammatory parameters have been used at re-evaluation to indicate the need for further treatment on a site-specific level. However, studies have challenged the usefulness of these signs. A deep residual probing depth at re-evaluation (⩾ 6 mm) may not indicate a failure of treatment as approximately 75% of these sites may show an improvement of ⩾ 1 mm at reevaluation compared to postinstrumentation baseline values (18).

Other studies, focusing on the predictive power of deep residual depths, reported weak predictive values of less than 30% for residual depths of ⩾ 7 mm (Fig. 4) (4, 20). These values increased to approximately 50% when residual depths from examinations

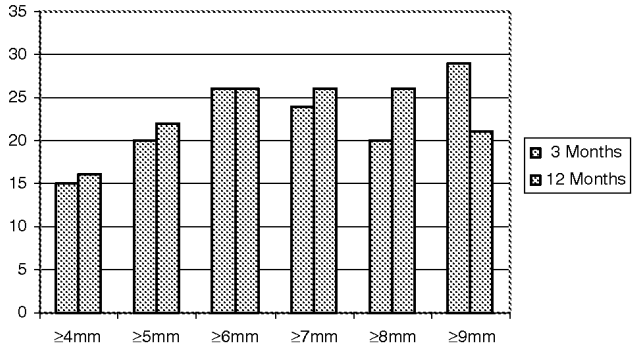

Fig. 4. Diagnostic predictability (%) of residual probing depth of different magnistude at 3 and 12 months for probing attachment loss at 42 months following nonsurgical therapy (Claffey et al. (20)).

further on in the maintenance (3.5–5 years) were evaluated. Likewise, increases in probing depth had poor to modest predictive values at short-term intervals following therapy. The predictive values for bleeding on probing evaluated from frequencies of examinations with bleeding on probing present during the first 12 months following initial therapy were also disappointing, with predictive values of <20%. When frequency of bleeding on probing was combined with either deep residual depth or increase in probing depth 3 years or longer after initial therapy, predictive values of 50–70% were found. Thus, it appears that the presence of these traditionally used signs at re-evaluation is of limited value in predicting ongoing attachment loss. On the other hand, the absence of bleeding has been demonstrated as a useful predictor of health (43).

It may be argued that individual highly skilled clinicians can predict deteriorating sites at re-evaluation on the basis of their own clinical judgment, the components of which are difficult to define. Vanooteghem et al. (83), reported predictive values of ⩾ 30% when the opinions of two of three experienced clinicians were used to denote sites susceptible to further deterioration.

There are limited data to suggest that higher percentages of deep residual probing depths at re-evaluation may be correlated with higher percentages of sites with ongoing attachment loss within patients (17).

In conclusion, it would appear that, on a site-specific level, the traditionally used clinical signs at reevaluation have limited diagnostic value in predicting future deterioration. These signs become more useful during prolonged periods of maintenance. On a patient level, the presence of high proportions of deep residual depths at re-evaluation may indicate patient susceptibility to further breakdown, although data to support this are limited.

Periodontal surgery

The following have been proposed as the aims of periodontal surgery:

- accessibility to previously inaccessible root surfaces.
- production of a healthy dentogingival junction that would enable the patient to practice a high level of plaque removal.
- reduction of probing depths to allow a) effectively delivered maintenance and home care and b) the monitoring and/or diagnosis of recurrent inflammation and progressive periodontal disease. (Modified from Friedman (27)).

These aims were later elaborated by Palmer & Floyd (61) to include correction of mucogingival deformities and the treatment of advanced periodontitis lesions that require reconstructive or regenerative therapy. For the purposes of this overview, the techniques discussed will be limited to pocket elimination and access procedures.

Pocket elimination was considered to be a desirable treatment outcome, giving rise to the gingivectomy and apically repositioned flap techniques.

Gingivectomy

The gingivectomy procedure was introduced by Robicsek in 1884 (74), and was later described by Grant et al. (31). This technique is aimed at reducing deep gingival pockets formed by enlarged fibrotic tissue and suprabony periodontal pockets. Gingivectomy is not indicated in the reduction of infrabony pockets because the incision cannot reach the base of the pocket due to intervening bone. Furthermore, if used for pockets extending to or beyond the mucogingival junction, the entire zone of keratinized gingiva is lost (30). Epithelialization of the gingivectomy wound starts within a few days following excision of the inflamed gingival soft tissues and is generally complete within 14 days after surgery. A new dentogingival unit is formed during the following weeks. Healing of the gingivectomy wound is completed within 4–5 weeks. However, on clinical examination the surface of the gingiva may already appear healed after 13–15 days (70).

The apically repositioned flap with or without osseous surgery

In 1954, Nabers (58) described the technique of 'repositioning of attached gingiva'. Friedman (27) later introduced the term 'apically repositioned flap' and stressed the importance of displacing the entire complex of soft tissues (gingival and alveolar mucosa) apically to achieve pocket elimination. The flap design often incorporates reverse bevel incisions and appropriately placed vertical incisions. Osseous surgery may be incorporated (osteoplasty or ostectomy) to reshape the alveolar bone crest to eliminate intrabony components of the pocket or to recapture the normal form of the alveolar process at a more apical level.

During the initial phase of healing, bone resorption of varying degrees takes place in the alveolar crest. A new dentogingival unit forms during the phase of tissue regeneration and maturation in a manner similar to that during healing following gingivectomy procedures (69).

In response to concerns about aesthetic and root sensitivity consequences of pocket elimination procedures, pocket reduction procedures were advocated as a more conservative and constructive surgical approach.

Modified Widman flap

The 'unrepositioned mucoperiosteal flap' or 'open flap curettage' was first described by Morris in 1965 (57) and later by Ramfjord & Nissle in 1974 (71). This procedure is more commonly used when the target of the surgery is to reduce the pocket depths through readaptation of periodontal tissues. It incorporates a conservative incision made 0.5–1.0 mm from the gingival margin and parallel to the long axis of the tooth. Two further incisions facilitate the separation of the soft tissue collar from the tooth and bone. Removal of granulomatous tissue and root planing is carried out, following which the flaps are sutured to the original marginal position with complete closure proximally (67). During healing some crestal bone resorption and osseous repair can be expected with the establishment of a 'long junctional epithelium' between the bone and the root surface (60, 87, 88). Barrington (5) suggested that healing following the Widman flap technique by means of a long junctional epithelium instead of a new connective tissue attachment may be a disadvantage and may lead to plaque infection and new pocket formation. In contrast, Magnusson et al. (54) reported in an animal study that a gingival unit with a long junctional epithelium does not provide a less efficient barrier against plaque infection when compared to dentogingival epithelium of normal length. Soft-tissue recession takes place during the healing phase and may continue for more than 1 year (46). More conservative procedures, such as the excisional new attachment procedure (ENAP), were also suggested. ENAP is defined as a subgingival curettage procedure carried out using a scalpel to remove the inner portion of the soft tissue wall of the periodontal pocket around the tooth. No attempt is made to raise a flap and all the connective tissue fibers that remain attached to the root surface are preserved (87, 88).

Comparison of surgical and nonsurgical treatment modalities

Most of the available studies comparing surgical with nonsurgical treatment have employed quadrant or split-mouth designs. When interpreting the results of these studies the following should be considered:

- Some studies were designed so that initial therapy, including full-mouth root planing, was carried out at the outset. Strictly speaking, what was then studied was an adjunctive effect of either further root planing or a surgical procedure.
- Results of probing measurements are often expressed as means of subgroups of sites, for instance, sites initially shallow, moderately deep and deep. However, mean improvements may mask individual site deterioration. Frequencies of sites with probing attachment loss/gain for various procedures are an important additional outcome variable.
- It is possible that periodontitis subjects, although untreated, would not display ongoing connective tissue loss during the observation interval of study. Thus comparison of different treatment modalities with the aim of arresting disease may not be meaningful. This may be particularly important in interpreting the results of shorter-term studies.
- Clinical results over short time periods of study may reflect more improvements due to changes in inflammation. Due to the generally slow progress of the disease process, allied with our relatively crude measurement techniques, longer observation intervals may be necessary to study the efficacy of therapies on arresting attachment loss.
- The vast majority of studies evaluating periodontal therapies lack an untreated control. This is perhaps to be expected, as ethical considerations restrict performance of such studies.

- If mean differences are reported as statistically significant, the clinical significance of such differences should be critically appraised.
- Assignation of quadrants to certain treatment modalities inevitably results in the application of clinically inappropriate techniques, for example treatment of shallow pockets with pocket elimination procedures.
- The subjects in many of these studies were attending University centres where compliance with recall and maintenance may not reflect that in practice generally.

An early study employing a split-mouth design was that of Knowles et al. (40). The subjects were given initial oral hygiene instruction and root planing. Three further modalities were tested; subgingival curettage (root planing combined with curettage of pocket lining); modified Widman flap surgery and pocket elimination surgery (either gingivectomy or apically repositioned flap with osseous surgery). Prophylaxis was carried out every 3 months together with oral hygiene reinforcement. For 8 years, annual recordings from five sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual and midlingual) were made. All techniques resulted in favorable changes in the means of the clinical parameters measured, although there was a slight tendency to relapse later on in the observation interval. The surgical techniques resulted in slightly more pocket reduction in deep pockets. Although all techniques yielded appreciable gains in clinical attachment in deep pockets, the modified Widman flap resulted in the greatest clinical attachment gain. In studies comparing the effects of root planing and modified Widman flap surgery over 6½ years of observation, the modified Widman flap resulted in more pocket reduction in initially deep pockets, although mean attachment levels were similar (63, 64).

Definitive scaling and root planing, inverse bevel flap and modified Widman flap were compared for their effects on 5-mm pockets over a 5-year period (34). The initial therapy provided a modest reduction in probing depth. Similar results in mean scores and frequencies of sites with probing attachment gain/loss were found for the modalities studied over the entire observation interval. A further study compared root planing, subgingival curettage, modified Widman flap and either gingivectomy or apically repositioned flap (68). Measurements were made before initial therapy (scaling/root planing/oral hygiene instruction) and 1 month following initial therapy but before the four experimental treatments were applied. Further treatment with all four modalities resulted in additional pocket reduction, although in general the improvements were less than those seen after the hygienic phase. In general, mean attachment values for deep pockets remained at the post-initial therapy levels for the 5 years of observation, although some mean deterioration was observed, principally during the 2nd year. These authors calculated frequencies of sites gaining and losing 2 mm and 3 mm at 5 years compared to baseline. Surprisingly, for all groups of sites subdivided by initial probing depth, little difference was seen in frequencies of sites deteriorating or gaining attachment. Tooth loss was also similar for the four modalities. It was interesting to note that maintenance treatment with supra- and subgingival debridement was judged necessary on about twice the number of teeth in the root planing category than on those in the other three categories.

All of the above mentioned studies featured an initial phase of treatment in which root debridement was performed. A further session of root planing as part of the experimental design was then carried out. To further study the effects of one session of root planing compared to surgical procedures, studies were designed in which the initial therapy was limited to oral hygiene instruction alone (47, 48). In general, both mean scores and frequencies of sites with gain/loss of attachment were similar for the two modalities, although molar teeth tended to display less favorable mean probing changes than nonmolar teeth, irrespective of treatment modality.

Coronal scaling alone was incorporated as a control into an experimental design in which modified Widman flap, apically repositioned flap including osseous surgery, and root planing were studied over a 7-year observation interval (35, 36). The coronal scaling modality had to be abandoned because of the high number of initially deep sites that progressed over the first years of study. There was somewhat more mean pocket reduction for moderately deep and deep sites initially but this tended to equalize later in the observation period. Changes in probing attachment levels were similar for the three modalities, with the exception of the more marked loss of attachment in shallow sites treated with repositioned flap including osseous surgery. Fewer sites lost probing attachment with the repositioned flap including osseous surgery modality than with the other modalities but this may have been associated to a greater or lesser extent with the higher number of presumably highly compromised teeth extracted and roots resected initially due to the impracticality of carrying out osseous surgery. Using the same study popula-

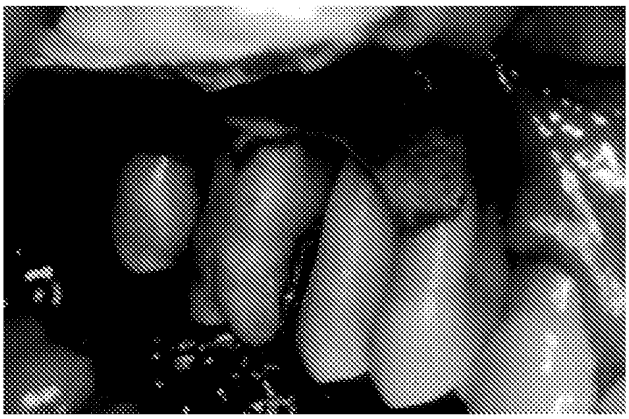

Fig. 5. It is paradoxical that surgical intervention following periodontal treatment is in widespread use despite equivocal data supporting the efficacy of such treatment.

tion as the study by Kaldahl et al. above, the same four modalities were studied for their effects on furcation sites over a 2-year observation interval. No major benefit was demonstrated for any one treatment method (37). A recent study compared scaling root planing, osseous surgery and modified Widman flap over 5 years (6). Scaling and root planing was delivered at the outset during two 1-h appointments but without the use of local anesthesia. Baseline data were gathered 3–4 weeks following initial therapy. Clinical outcomes were similar for the three methods. The authors place emphasis on the importance of effective maintenance irrespective of whatever technique is considered clinically appropriate for use in the circumstances prevailing.

In summary, the following points may be deduced from appraisal of the available literature (Fig. 5):

- Both nonsurgical and surgical therapies have been shown to result in similar mean improvements of clinical scores, which, in general, suggests stability in attachment levels following therapy.
- Data for the possible adjunctive effect of surgical procedures on patients / sites unresponsive to initial therapy are scarce.
- Data for the possible adjunctive effect of surgical procedures on patients believed to be at high risk to ongoing attachment loss are scarce.
- Other than studies on regenerative techniques, data for the comparable effects of different surgical modalities on furcation areas are also scarce.
- The literature reviewed herein cannot be used for comparison of treatment modalities on the less common forms of periodontitis, for example aggressive periodontitis.
- Data for long-term outcome measures, such as tooth loss and quality of life issues, are scarce.

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Immunoglobulin G and A Antibody Responses to *Bacteroides forsythus* and *Prevotella intermedia* in Sera and Synovial Fluids of Arthritis Patients

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The objective of the present study was to investigate immunoglobulin G (IgG) and IgA antibody immune responses to *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, and *Candida albicans* in the sera of patients with rheumatoid arthritis (RA), the synovial fluid (SF) of patients with RA (RA-SF samples), and the SF of patients without RA (non-RA-SF samples). An enzyme-linked immunosorbent assay was used to determine IgG and IgA antibody levels in 116 serum samples from patients with RA, 52 RA-SF samples, and 43 non-RA-SF samples; and these were compared with those in SF samples from 9 patients with osteoarthritis (OA-SF samples) and the blood from 100 donors (the control [CTR] group). Higher levels of IgG antibodies against *B. forsythus* ($P < 0.0001$) and *P. intermedia* ($P < 0.0001$) were found in non-RA-SF samples than in OA-SF samples, and higher levels of IgG antibodies against *B. forsythus* ($P = 0.003$) and *P. intermedia* ($P = 0.024$) were found in RA-SF samples than in OA-SF samples. Significantly higher levels of IgA antibodies against *B. forsythus* were demonstrated in both RA-SF and non-RA-SF samples than in OA-SF samples. When corrected for total Ig levels, levels of IgG antibody against *B. forsythus* were elevated in RA-SF and non-RA-SF samples compared to those in OA-SF samples. Lower levels of Ig antibodies against *B. forsythus* were found in the sera of patients with RA than in the plasma of the CTR group for both IgG ($P = 0.003$) and IgA ($P < 0.0001$). When corrected for total Ig levels, the levels of IgG and IgA antibodies against *B. forsythus* were still found to be lower in the sera from patients with RA than in the plasma of the CTR group ($P < 0.0001$). The levels of antibodies against *P. gingivalis* and *C. albicans* in the sera and SF of RA and non-RA patients were comparable to those found in the respective controls. The levels of IgG and IgA antibodies against *B. forsythus* were elevated in SF from patients with RA and non-RA-SF samples compared to those in OA-SF samples. Significantly lower levels of IgG and IgA antibodies against *B. forsythus* were found in the sera of patients with RA than in the plasma of the CTR group. This indicates the presence of an active antibody response in synovial tissue and illustrates a potential connection between periodontal and joint diseases.

Sufficient data are available to implicate *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Prevotella intermedia* as pathogens that initiate periodontal disease (2, 40, 43). These gram-negative anaerobic bacteria possess various antigens (32, 36) that provoke a host-mediated immune response to the offending species (2, 36). This is a complex immunopathogenic process which involves interactions between T and B lymphocytes, neutrophils, monocytes, and phagocytes and the subsequent production of cytokines and prostaglandins (15). The humoral immune response, in which immunoglobulin G (IgG) and IgA antibodies are produced, is considered to have a protective role in the pathogenesis of periodontal disease (2, 13, 22), but the mechanisms are not fully understood.

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by synovial hyperplasia and chronic inflammation (14). Peripheral joint disease is the most frequent feature of RA; but the eyes, skin, blood vessels, kidneys, and nervous system may also be involved. The prevalence of RA in the Western population is 0.5 to 1% and affects women about three times as often as it affects men (35). Experimental evidence has suggested that several microorganisms (bacterial proteins or viruses) may play an important role, in association with a genetic predisposition (3), in triggering the onset of the disease (25, 31, 44).

Clinical studies of RA and periodontal disease have provided evidence for a significant association between the two disorders (29). Patients with long-standing active RA have a substantially increased frequency of periodontal disease compared to that among healthy subjects (21). Patients with periodontal disease have a higher prevalence of RA than patients without periodontitis (28), and it may be hypothesized that periodontal disease plays a role as a triggering factor for RA.

Dry eyes and dry mouth have been shown to be major complaints among RA patients, but the prevalence is uncertain (4, 10, 11, 38). Decreased salivary secretion also affects the oral mucosa and may promote oral candidiasis (18). Interestingly, it has been shown that RA patients may have higher counts of oral *Candida* species than controls (19).

The last few decades of periodontal research have provided evidence for a correlation between elevated concentrations of antibodies against periodontal pathogens in serum and disease severity (2, 13, 22, 42). However, few studies that have attempted to search for a link between oral microorganisms and immune responses in the sera of patients with RA (RA sera)

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TABLE 1. Levels of IgG antibodies against *P. gingivalis*, *P. intermedia*, and *B. forsythus* in RA and CTR group sera

Sample	No. of samples	Mean (SD) IgG level (μg/ml)			Mean (SD) total IgG level (mg/ml)
		P. gingivalis	*P. intermedia*	*B. forsythus*	
RA serum	116	9.85 (12.08)	2.21 (1.72)	3.92[a] (3.34)	26.50[b] (10.04)
CTR	100	7.70 (9.99)	1.91 (1.10)	4.65 (2.96)	19.68 (8.29)

[a] $P < 0.01$ for differences in antibody levels between the patient group (RA serum) and the CTR group (CTR plasma) calculated by the Mann-Whitney U test.
[b] $P < 0.0001$ for differences in antibody levels between the patient group and the CTR group calculated by the Mann-Whitney U test.

and synovial fluid (SF) samples from patients with RA (RA-SF samples) have been carried out. In a study of cell wall components from the periodontal pathogen *Actinobacillus actinomycetemcomitans*, it was shown that IgG antibody titers were significantly higher in RA sera than in the sera of healthy controls (41). Other findings suggest that while the levels of IgG antibodies against *P. gingivalis* were significantly elevated in sera from patients with periodontal disease, comparable levels were found in the sera of healthy controls and RA patients (42).

In order to further elucidate the role of periopathogens in RA, the purpose of this study was to screen for the levels of IgG and IgA antibodies against *P. gingivalis*, *P. intermedia*, *B. forsythus*, and *Candida albicans* in serum and RA-SF samples and samples from other arthritis patients compared to those in patients with osteoarthritis (OA) and healthy subjects.

MATERIALS AND METHODS

Patients and sampling. All rheumatic patients were examined at the Rheumatology Clinic at Haukeland University Hospital, Bergen, Norway. A medical history was taken from each patient. Recorded serological data included rheumatoid factor (RF) titers and C-reactive protein (CRP) levels. CRP levels were determined by endpoint analysis (Tina Quant method) at a central laboratory, Haukeland University Hospital, during hospitalization. The cutoff level for a positive RF titer was set as a titer of 128 (11, 39), as determined by the hemagglutination method. The study was approved by the Ethical Committee, Faculty of Medicine, University of Bergen.

Group 1 consisted of 116 patients with RA. Eighty-two (70.7%) of these patients were women, and 34 (29.3%) were men. The mean ages were 58.6 years (range, 33 to 80 years) for women and 60.4 years (range, 33 to 80 years) for men. Ninety-two (79.3%) were treated with disease-modifying antirheumatic drugs (DMARDs), and the most frequent drug administered was methotrexate (MTX), which was administered to 63 patients. Steroids were given orally to 42 (36.2%) of the patients. The mean duration of DMARD treatment in this group was 7.0 years (range, 0 to 27 years).

Group 2 consisted of 104 patients who were different from those in group 1. Fifty-six (53.8%) of these patients were women, and 48 (46.2%) were men, and all had some type of inflammatory arthritis. Among these, 52 patients (34 women, 18 men) had RA, 9 (5 women, 4 men) had OA, and 43 (16 women, 27 men) had various arthritides (non-RA). Among the patients in the non-RA group, 14 had psoriatic arthritis, 10 had morbus Bechterew, 9 had reactive arthritis, 2 had morbus Reiter, and 8 were characterized as having arthritis in connection with ulcerative colitis and nonspecific mono- or polyarthritis. The mean ages for the RA patients were 53.0 years (range, 23 to 86 years) for women and 60.3 years (range, 20 to 76 years) for men. For the OA patients, the mean ages were 37.9 years (range, 13 to 68 years) for women and 43.1 years (range, 22 to 78 years) for men, and for the non-RA patients the mean ages were 57.2 years (range, 54 to 58 years) for women and 59.5 years (range, 52 to 68 years) for men. SF samples from OA patients (OA-SF samples) were used as controls (see Tables 2, 4, and 5).

Twenty-seven (53.0%) of the patients in group 2 were medically treated with DMARDs, and 14 of them were treated with MTX. Steroids were administered orally to 22 patients. One OA patient who provided an SF sample and three non-RF patients who provided an SF sample were treated with MTX. Steroids were given orally to four of the non-RA patients who provided an SF sample.

All RA patients fulfilled the revised criteria of the American College of Rheumatology (formerly the American Rheumatism Association) (7). Peripheral blood samples were collected from RA patients in group 1 at the time of medical examination and were immediately centrifuged, and the sera were stored at −20°C.

SF, mainly from larger joints, was obtained only from patients in group 2, centrifuged, and stored at −20°C.

Group 3 consisted of 100 healthy blood donors (71 women, 29 men) who were matched by gender and age with the patients in group 1 and who had no history of active rheumatic disease. The mean ages were 60.4 years (range, 41 to 70 years) for women and 62.3 years (range, 33 to 80 years) for men. The plasma was stored at −20°C and served as the control (CTR) group. In this study blood donor plasma was found to fulfill the criteria as controls for RA sera.

Microbial antigens used in enzyme-linked immunosorbent assay (ELISA). *P. gingivalis* ATCC 33277 and *P. intermedia* ATCC 25611 were obtained from the American Type Culture Collection (ATCC), Manassas, Va. Both were cultured in Lab 090-A Fastidious Anaerobic Agar (LabM, IDG [United Kingdom] Limited, Bury, England), to which sheep blood was added.

B. forsythus FDC 2008 was obtained from the Forsythe Dental Center, Boston, Mass., and was cultured in Trypticase soy agar with *N*-acetylmuramic acid stock solution.

Mannan (M7504) was obtained from Sigma Chemical Company, St. Louis, Mo. The powder was diluted in distilled water so that the concentration of the solution was 27.2 mg/ml.

The organisms were cultivated in an anaerobic glove box for 10 days at 35°C and then harvested and recultivated for 10 additional days. The second-generation bacterial cultures were inactivated in 1% formalin for 24 h, washed twice in phosphate-buffered saline (PBS), and centrifuged at 10,000 × *g* for 10 min. The inactivated whole cells were again washed and diluted in PBS to approximately $1{,}500 \times 10^6$ cells/ml of PBS solution by comparison with McFarland standards (24).

ELISAs for IgG and IgA antibody detection. The concentrations of IgG and IgA antibodies against whole-cell *P. gingivalis*, *P. intermedia*, and *B. forsythus* and the *C. albicans* polysaccharide mannan in RA sera, CTR plasma, and SF were determined by ELISA with 96-well microtiter flat-bottom high-binding plates (Costar, Cambridge, Mass.) (12, 16). All analyses were carried out in duplicate and were performed with 100 μl of solution per well.

The plates were coated overnight at 4°C with suspensions of inactivated whole bacterial cells ($1{,}500 \times 10^6$ cells/ml of PBS).

For IgG antibody determinations, the suspensions were 1/100 for *P. gingivalis* and *B. forsythus* and 1/80 for *P. intermedia*. *C. albicans* mannan was diluted to 5 μg/ml in 0.05 M carbonate buffer. For IgA antibody determination, the suspensions were diluted 1/100 in PBS for *P. gingivalis*, *P. intermedia*, and *B. forsythus*. *C. albicans* mannan was diluted to 5 μg/ml in 0.05 M carbonate buffer.

All plates were washed four times with PBS–0.05% Tween 20 (PBST) and were then blocked with PBST–5% milk (L 31 skim milk; Oxoid, Basingstoke, England) for 1 h at room temperature.

For specific IgG antibody determinations, RA sera and CTR plasma were diluted 1/100 for *P. gingivalis* and *P. intermedia*, while SF was diluted 1/10 for *P. gingivalis* and *P. intermedia*. For *B. forsythus* and *C. albicans* mannan the RA sera and the CTR plasma were diluted 1/50 and SF was diluted 1/40. The RA sera, CTR plasma, and SF samples were diluted 1/10 for specific IgA antibody determinations. IgG and IgA antibody standards (3.125 to 200 ng/ml) were prepared by diluting purified human IgG (1.274 mg/ml; I4506; Sigma) and human IgA (1 mg/ml; I1010; Sigma) in PBST–5% milk.

After 1.5 h at room temperature, the plates were washed four times with PBST. Peroxidase-conjugated goat anti-human IgG (A0293; Sigma) was diluted 1/2,000 in PBST–5% milk for all plates except those for *P. intermedia*, which were diluted 1/1,500 for RA sera and CTR plasma, and was then added to the plates. Peroxidase-conjugated goat anti-human IgA (A7032; Sigma) was diluted 1/3,000 for all plates.

TABLE 2. Specific levels of IgG antibodies against *P. gingivalis*, *P. intermedia*, and *B. forsythus* in SF

Sample	No. of samples	Mean (SD) IgG level (μg/ml)			Mean (SD) total IgG level (mg/ml)
		P. gingivalis	*P. intermedia*	*B. forsythus*	
RA-SF	52	1.43 (1.08)	0.82[a] (0.525)	1.51[b] (1.309)	8.80[b] (4.18)
Non-RA-SF	43	1.56 (0.641)	0.96[c] (0.401)	1.68[c] (1.156)	11.61[c] (7.44)
OA-SF	9	1.36 (0.519)	0.47 (0.056)	0.45 (0.118)	4.22 (1.13)

[a] $P < 0.05$ for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.
[b] $P < 0.01$ for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.
[c] $P < 0.0001$ for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.

After 1 h at room temperature the plates were washed four times and substrate was added. 1,2-Phenylenediamine dihydrochloride tablets (Dako, Glostrup, Denmark) were dissolved in water by the addition of H_2O_2. The reaction was stopped with 1 M H_2SO_4 and read at 490 nm with an E-max precision microplate reader (Molecular Devices Corporation, Sunnyvale, Calif.). The software SOFTmax (Molecular Devices Corporation) was used to calculate the values. Prior to all assays, pilot studies were performed with a limited number of randomized selected samples to find the most appropriate dilutions.

Total IgG and IgA levels were determined by coating the plates with goat anti-human IgG (1 mg/ml; I3382; Sigma) and goat anti-human IgA (3 mg/ml; I1261; Sigma) diluted 1/2,000 and 1/4,000 in PBS, respectively. IgG and IgA antbody standards (3.125 to 200 ng/ml) were prepared by diluting purified human IgG (1.274 mg/ml; I4506; Sigma) and human IgA (1 mg/ml; I1010; Sigma) in PBST–5% milk. The RA serum, CTR plasma, and SF samples were diluted $1/10^6$ in PBST–5% milk. Peroxidase-conjugated goat anti-human IgG (A0293; Sigma) and peroxidase-conjugated goat anti-human IgA (A7032; Sigma) were diluted 1/2,000 and 1/3,000 in PBST–5% milk, respectively. The ELISA was performed as described above for the bacterial suspensions.

Calculation of antibody standard. The plates were coated with the whole bacterial suspensions. After some of the sera were screened, one sample with high levels of IgG antibodies (human serum) was used as the reference standard. This serum sample was assigned a value of arbitrary units by endpoint titration. In the subsequent analysis, twofold serial dilutions of this serum sample were added to each ELISA plate. In one plate some wells were coated with goat anti-human IgG (1 mg/ml; I3382; Sigma), and then a known amount (in nanograms per milliliter) of purified human IgG (1.274 mg/ml; I4506, Sigma) was added. Some other wells of the same plate were coated with bacteria, and then the reference serum sample was added. The same amount of peroxidase-conjugated goat anti-human IgG (A0293; Sigma) was added to the plate. The IgG titers for the human serum was read against the known IgG values. The units were translated to nanograms per milliliter.

Antibody specificity. The human serum was mixed with *P. gingivalis* cells (1 part diluted serum [1/50] and two parts *P. gingivalis* [1.5×10^9 cells/ml]). The mixture was shaken on a Schüttler MTS 4 shaker (IKA) for 4 h at room temperature and was then left overnight at room temperature. After centrifugation the supernatant was tested by ELISA with *P. gingivalis*, *P. intermedia*, and *B. forsythus* as antigens. Anti-human IgG (I3382; Sigma) served as coat for standard IgG (I4506; Sigma). ELISA was performed as described above with peroxidase-conjugated goat anti-human IgG (A0293; Sigma).

Statistical analysis. Statistical analysis of the differences between the patient groups and the controls was carried out by the Mann-Whitney U test. The level of significance was set at a P value of <0.05. The associations between specific antibody levels and total IgG and IgA levels were analyzed by use of Spearman's rho correlation coefficient. The statistical analyses were performed with SPSS software (release 10.0.0; SPSS Inc., Chicago, Ill.).

RESULTS

Total Ig levels. The total IgG antibody levels were significantly higher in RA sera than in CTR plasma ($P < 0.0001$) (Table 1). Total IgG levels were also higher in RA-SF samples ($P = 0.001$) and SF samples from non-RA patients (non-RA-SF samples) ($P < 0.0001$) than in SF samples from patients with OA (OA-SF samples) (Table 2). No differences in total IgA antibody levels in sera were seen (Table 3), but significantly higher IgA antibody levels were found in RA-SF ($P = 0.001$) and non-RA-SF ($P < 0.0001$) samples than in OA-SF samples (Table 4).

Specific IgG and IgA antibody levels. RA sera contained significantly lower levels of antibodies against *B. forsythus* than CTR plasma (Tables 1 and 3). The P values for differences in IgG antibody levels were 0.003 and <0.0001 for IgA, respectively. In contrast, the levels of IgG antibodies against *B. forsythus* were significantly higher in both RA-SF ($P = 0.003$) and non-RA-SF ($P < 0.0001$) samples than in OA-SF samples (Table 3). Significantly higher levels of IgA antibodies against *B. forsythus* were also seen in RA-SF ($P = 0.027$) and non-RA-SF ($P < 0.0001$) samples than in OA-SF samples (Table 4).

The levels of IgG antibodies against *P. intermedia* were significantly higher in both RA-SF ($P = 0.024$) and non-RA-SF ($P < 0.0001$) samples than in OA-SF samples (Table 2). Similarly, non-RA-SF samples had stronger antibody responses against *B. forsythus* and *P. intermedia* than RA-SF samples.

The levels of IgA antibodies against *P. intermedia* in RA sera and SF samples and against *P. gingivalis* in SF samples were below the lowest standard value (<3.125 ng/ml) and were excluded as a result of the findings from the pilot studies with five randomly selected samples (Tables 3 and 4).

Although the levels of IgG and IgA antibodies against *P. gingivalis* tended to be higher in RA serum and SF samples than in the plasma from the CTR group, the differences were not statistically significant (Tables 1 to 3).

TABLE 3. Levels of specific IgA antibodies against *P. gingivalis*, *P. intermedia*, and *B. forsythus* in RA and CTR sera

Sample	No. of samples	Mean (SD) IgA level (μg/ml)			Mean (SD) total IgA level (mg/ml)
		P. gingivalis	*P. intermedia*	*B. forsythus*	
RA-serum	116	0.169 (0.176)	Not detectable[a]	0.349[b] (0.275)	2.33 (1.50)
CTR	100	0.129 (0.121)	Not detectable	0.532 (0.390)	2.28 (2.06)

[a] Not detectable, values less than the lowest standard value used.
[b] $P < 0.0001$ for differences in antibody levels between the patient group (RA serum) and the CTR group (CTR plasma) calculated by the Mann-Whitney U test.

TABLE 4. Levels of IgA antibodies against *P. gingivalis*, *P. intermedia*, and *B. forsythus* in SF

Sample	No. of samples	Mean (SD) IgA level (μg/ml)			Mean (SD) total IgA level (mg/ml)
		P. gingivalis	*P. intermedia*	*B. forsythus*	
RA-SF	52	Not detectable[a]	Not detectable	0.275[b] (0.246)	1.32[c] (0.803)
Non-RA-SF	43	Not detectable	Not detectable	0.501[d] (0.440)	1.59[d] (0.949)
OA-SF	9	Not detectable	Not detectable	0.100 (0.071)	0.47 (0.575)

[a] Not detectable, values less than the lowest standard value used.
[b] $P < 0.05$ for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.
[c] $P < 0.01$ for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.
[d] $P < 0.0001$ for differences in antibody levels between each patient group and controls (OA-SF samples) calculated by the Mann-Whitney U test.

Higher levels of IgG antibodies against *C. albicans* mannan were seen in RA sera than in CTR plasma, although these differences were not significant ($P = 0.072$). The differences in IgG antibody levels in RA-SF and non-RA-SF samples compared to those in OA-SF samples were not statistically significant, and the detectable IgA levels were below the lowest standard value.

Total Ig and specific antibody levels. Significant associations between specific antibodies and the total Ig levels in serum and SF samples were detected. In RA serum (Fig. 1 and 2), total IgG antibody levels correlated with the levels of IgG antibodies against *P. intermedia* ($r = 0.251$; $P = 0.007$) and *B. forsythus* ($r = 0.249$; $P = 0.007$). No significant correlation was found between the levels of IgG antibodies against *P. gingivalis* and the total IgG level in RA sera.

A significant correlation between the total IgA antibody level and the *B. forsythus*-specific IgA level was evident in RA serum ($r = 0.198$; $P = 0.033$).

A significant correlation between the levels of IgG antibodies against *P. intermedia* and total IgG levels in RA-SF samples was present ($r = 0.519$; $P < 0.0001$) (Fig. 3). Increased levels of IgG ($r = 0.372$; $P = 0.007$) and IgA ($r = 0.588$; $P < 0.0001$) antibodies against *B. forsythus* correlated with increased total IgG and IgA antibody levels in RA-SF samples. No significant relation between the total antibody levels and specific Ig antibody levels in non-RA-SF samples was shown. Significant relations between total antibody levels and *C. albicans* mannan specific antibodies were absent for RA serum, CTR plasma, and SF samples.

When corrected for the total Ig levels (Ig level/total Ig level), lower levels of IgG and IgA antibodies against *B. forsythus* in RA sera than in CTR plasma ($P < 0.0001$) were demonstrated (Table 5). Although the differences were not significant ($P = 0.120$), a tendency toward higher levels of IgG antibodies against *B. forsythus* in the non-RA-SF and RA-SF samples than in OA-SF samples was seen. For IgA this tendency was also evident in non-RA-SF samples (Table 5). Minor differences were detected for *P. intermedia*.

Serological data and antibody levels. The sera of 63 (54.3%) of the RA patients were RF positive, and significant relations between RF titers and total IgA antibody levels ($r = 0.698$; $P < 0.0001$) and between RF titers and the levels of IgG antibodies against *P. gingivalis* ($r = 0.189$; $P = 0.043$) were found. No significant associations between RF titers and total IgG levels or between RF levels and the levels of specific Ig against the other bacteria and *C. albicans* were seen.

The SF of 16 (32%) of the 50 RA patients was RF positive, and a significant correlation between RF levels and total IgA antibody levels was found ($r = 0.449$; $P = 0.001$). The SF of all of the non-RA patients was RF negative, and the SF of one of

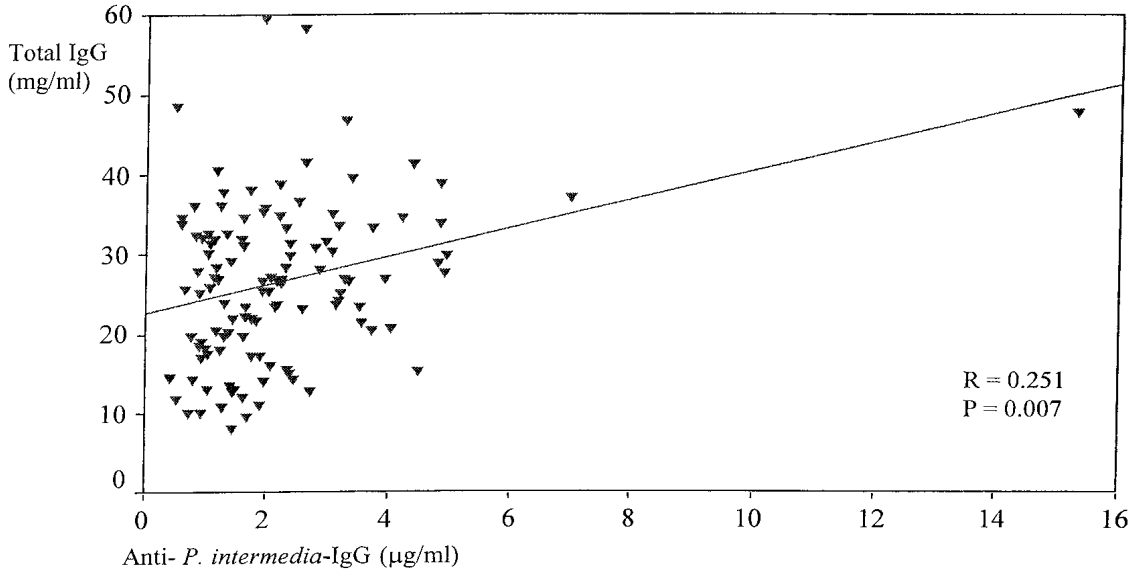

FIG. 1. Relation between levels of IgG antibodies against *P. intermedia* and total IgG levels in RA serum.

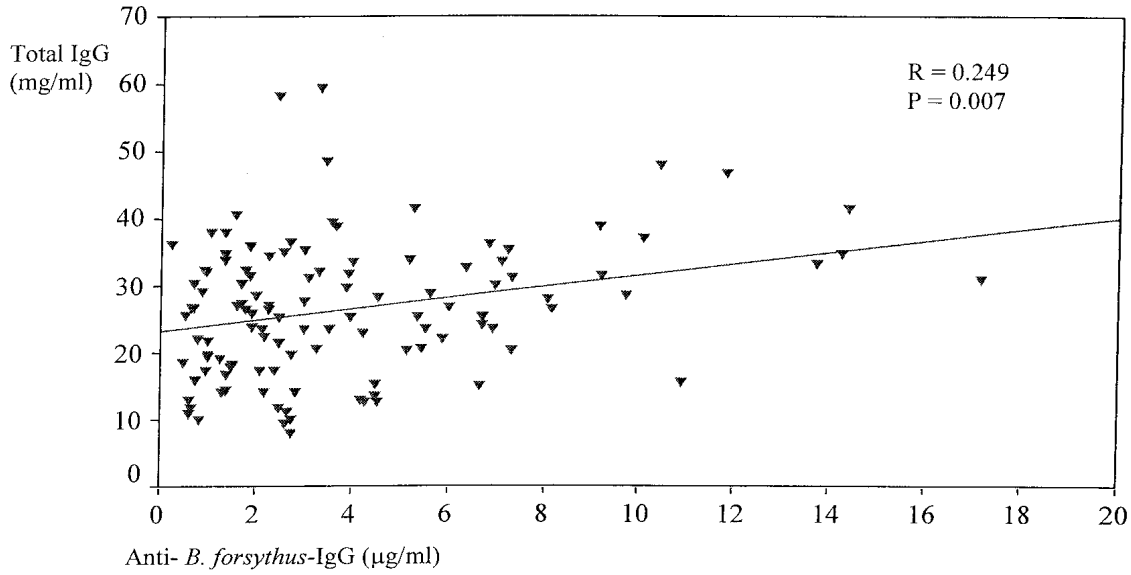

FIG. 2. Relation between levels of IgG antibodies against *B. forsythus* and total IgG levels in RA serum.

the OA patients was RF positive. No significant correlation was seen between RF titers and total IgG antibody levels or between RF levels and the levels of Ig against the other bacteria and *C. albicans* in SF.

The median CRP level among the serum samples from RA patients was 20.8 mg/liter (range, 0 to 139 mg/liter). A significant association between CRP and total IgG levels ($r = 0.291$; $P = 0.002$) was found. Positive associations between CRP levels and both the levels of IgG antibodies against *B. forsythus* ($r = 0.187$; $P = 0.047$) and the levels of IgG antibodies against *P. intermedia* ($r = 0.227$; $P = 0.016$) were also demonstrated.

The mean CRP level for the RA-SF group was 52.0 mg/liter (range, 2.0 to 122.0 mg/liter), that for the OA-SF group was 5.8 mg/liter (range, 4.0 to 9.0 mg/liter), and that for the non-RA-SF group was 43.4 mg/liter (range, 0 to 240 mg/liter). For the RA-SF group, there was a significant correlation between CRP levels and the levels of IgA antibodies against *B. forsythus* ($r = 0.302$; $P = 0.037$) and between CRP levels and total IgA levels ($r = 0.489$; $P < 0.0001$).

Antigen specificity. Testing of the antigen specificity between *P. gingivalis* and the other two bacteria showed that 70% of the anti-*P. gingivalis* IgG antibodies did not react when they were mixed with the *P. intermedia* suspension and that 77% of the anti-*P. gingivalis* IgG antibodies did not react when they were mixed with the *B. forsythus* suspension.

DISCUSSION

In this study we have shown that there is a different immune response against some selected periodontal pathogens in se-

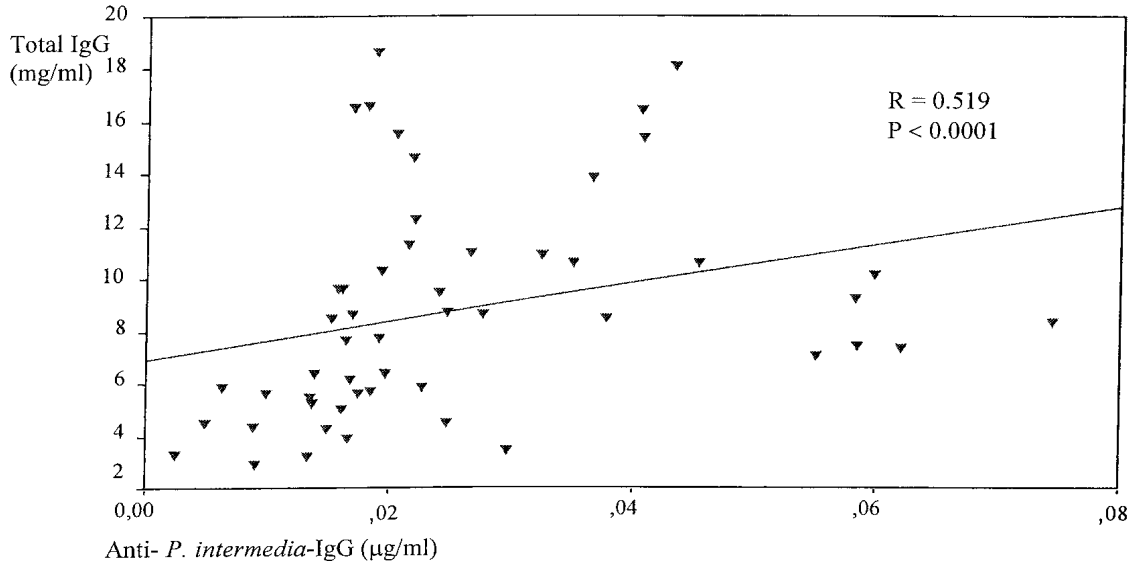

FIG. 3. Relation between levels of IgG antibodies against *P. intermedia* and total IgG levels in RA-SF samples.

TABLE 5. Levels of antibodies against *B. forsythus* and *P. intermedia* corrected for total Ig levels

Sample	No. of samples	Mean (SD) ratio (10^5) of IgG level/total IgG level		Mean (SD) ratio (10^5) of IgA level/total IgA level	
		B. forsythus	*P. intermedia*	*B. forsythus*	*P. intermedia*
RA serum	116	15.7[a] (12.4)	8.90[b] (5.5)	20.6[a] (23.0)	Not detectable[c]
Control plasma	100	28.3 (23.2)	12.4 (11.1)	28.9 (22.0)	Not detectable
RA-SF	52	18.3 (14.4)	10.3 (6.7)	22.2[b] (16.6)	Not detectable
Non-RA-SF	43	19.7 (17.5)	11.3 (7.9)	40.1 (39.5)	Not detectable
OA-SF	9	11.7 (5.0)	11.8 (5.9)	32.4 (12.8)	Not detectable

[a] $P < 0.0001$ for differences in antibody levels between the patient group (RA serum) and the CTR group (CTR plasma) and between RA-SF, non-RA-SF, and OA-SF samples calculated by the Mann-Whitney U test.
[b] $P < 0.05$ for differences in antibody levels between the patient group (RA serum) and the CTR group (CTR plasma) and between RA-SF, non-RA-SF, and OA-SF samples calculated by the Mann-Whitney U test.
[c] Not detectable, values less than the lowest standard value used.

rum and SF between arthritis patients and controls. Significantly higher levels of IgG and IgA antibodies against *B. forsythus* and *P. intermedia* were found in SF from RA- and non-RA-patients than in SF from OA patients. However, the levels of antibodies against *P. gingivalis* in RA sera were comparable to those found in the plasma of the CTR group, which has also been demonstrated by others (42). These findings could indicate that a stronger activation of specific B cells with the subsequent formation of antibodies directed especially against *B. forsythus* and *P. intermedia* is present in the joints of RA patients and patients with other arthritides.

The lower total antibody levels in SF compared to those in serum, findings which are in agreement with those of other investigators (27), imply that this immune response predominantly takes place in the surrounding tissue and to a lesser degree in the joint cavity. A possible mechanism for this is the capture of bacterial DNA or bacterial products in the synovial tissue, where they promote proinflammatory responses. Plasma cells may also be recruited nonspecifically to the joint. Higher mean levels of Ig antibodies (Tables 1 to 5) against *B. forsythus* compared to the levels of antibodies against *P. intermedia* were found. This and the fact that only IgA antibodies against *B. forsythus* could be detected in SF support the assumption of a particularly strong synovial immune response to *B. forsythus*.

We also demonstrated significantly lower levels of IgG and IgA antibodies against *B. forsythus* in RA serum than in the plasma of the CTR group. Given the higher SF antibody concentrations, it could be assumed that the B-cell homing mechanism toward synovial tissue is more pronounced in RA patients and patients with other arthritides and that this homing mechanism is particularly strengthened for *B. forsythus*. It has also been demonstrated that mucosal lymphocytes in particular are able to home to the synovium by expression of relevant adhesion molecules (8). The explanation why the levels of antibodies against this pathogen were particularly lower in RA serum is disputable. However, studies have shown reduced systemic levels of IgG against peptidoglycans from intestinal bacteria, indicating insufficient protection from spreading peptidoglycans, which was hypothesized to contribute to inflammatory processes (30).

To the best of our knowledge, our study is the first to investigate the role of periodontal pathogens in SF from RA patients and patients with other arthritides. Previous studies have focused on the role of antibody levels in the sera of patients with periodontal disease (2, 13, 22, 42). Researchers have also been interested in the role of the concentrations of antibodies against periodontal pathogens in the serum of RA patients and have investigated the relationship between periodontal disease and RA (21, 28, 29, 33, 41, 42). However, very few studies have been able to provide clinical periodontal data as a supplement to the immune status. Periodontal status has been shown to vary between RA patients and controls, and it is assumed that patients with long-standing active RA have an increased frequency of periodontal disease (21). Our findings regarding the mean antibody levels (Tables 1 to 5) could support this assumption that the incidence of periodontal disease could be higher in RA patients and patients with other arthritides than in healthy blood donors and OA patients, respectively.

Many similarities between the immunopathologies of RA and periodontal disease have been detected (29, 41, 42). Both are destructive, chronic, and inflammatory conditions and may have an acute or chronic presentation. Periodontitis affects single or multiple sites within the oral cavity, leading to the loss of the supporting periodontal tissue (23). In parallel with the pathogenesis seen in RA, the degenerative capability of periodontitis may be initiated and maintained by the presence of endotoxins and exotoxins introduced by microbial organisms (13, 32, 44). It is suggested that while T lymphocytes and polymorphonuclear cells dominate in the early stages of plaque accumulation and gingivitis, B cells dominate in more advanced lesions, with a higher degree of soft tissue destruction and bone loss (9, 36). As is seen in periodontal disease (5, 15, 20, 36), B cells are activated in the synovial tissue in patients with arthritis, leading to a spontaneous secretion of Igs (IgG, IgA, and IgM) (1). This lymphocytic response may lead to the subsequent formation of immune complexes, which are capable of inducing a spectrum of inflammatory mediators (17, 34). In patients with periodontal disease, inflammatory mediators are connected to tissue destruction and bone loss (15).

The cell wall polysaccharide mannan is considered a major antigen of the yeast *C. albicans* and has the ability to stimulate specific cell-mediated and humoral IgG and IgA immune responses (26). It has been shown that mannan antibodies more frequently appear in sera from patients with Crohn's disease and ulcerative colitis than in controls (6). In our study, we did not find any significant differences in seroreactivity to *C. albi-*

cans between RA sera and the plasma of the CTR group or between RA-SF, non-RA-SF, and OA-SF samples. This could indicate that the activation of B cells against *C. albicans* in serum or synovial tissue is a rare event.

In this study we found that high levels of antigen-specific antibodies against periodontal pathogens weakly correlated with the total IgG and IgA antibody levels (Fig. 1 and 2). However, when the levels were corrected for total IgG levels (Table 5), lower IgG antibody levels in RA serum compared to those in CTR plasma and higher levels in RA-SF and non-RA-SF samples compared to those in OA-SF samples still predominated. Corrected values for the non-RA-SF group supported the initial findings of the stronger generation of IgA against antibodies *B. forsythus* in the SF of non-RA patients than in the SF of the OA patients (Table 5). These results are further strengthened by the fact that CRP levels correlated significantly with the levels of IgA antibodies against *B. forsythus* in the RA-SF group.

Considering the mean age differences in the non-RA-SF group compared to the RA-SF group and the OA-SF group, it might also be suggested that the immune response is stronger in younger patients.

The RF titers did not seem to correlate with the Ig levels against the different bacteria. These findings demonstrate that elevated levels of Ig antibodies against periodontal bacteria in arthritis patients are independent of an up-regulated immune response in general and underline the initial assumption of elevated levels of antibodies against *P. intermedia* and *B. forsythus* in the RA-SF and non-RA-SF samples compared to those in the OA-SF samples.

Research regarding the role of intestinal bacteria and the connection between arthritis and bacterial products or bacterial DNA has been presented (30, 37). In light of this, one might assume that our results elucidate an association between oral microbial flora and arthritis. The higher levels of antibodies against these bacteria in arthritis patients demonstrate that they have a higher affinity for the antigens possessed by the cell membranes of periodontal pathogens than those of antibodies in other patient groups. This could be due to earlier exposure to bacterial antigens or products in certain regions such as the synovium or connective tissue as a result of shared antigens between related species in the oral and the intestinal floras. More research should be carried out in this field to elucidate the similarities between oral and intestinal pathogens and their possible arthritogenic capacities.

In conclusion, we have demonstrated evidence for raised levels of antibodies against some selected periodontal pathogens in the serum and intra-articular space of patients with different forms of arthritis, with a special focus on RA. Although the levels of antibodies were significantly higher against *P. intermedia* and *B. forsythus* in serum and SF samples from RA patients than in SF samples from OA patients, we demonstrated that the levels of antibodies against *P. gingivalis* were only slightly elevated. This could indicate a potential connection between periodontal and joint diseases, and our data should lead to more studies in order to evaluate the effect of oral microorganisms in relation to the pathogenesis of RA and related arthritis disorders.

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Modulation of the innate immune response within the periodontium

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Within the periodontium, the characteristic shift in microbial compositions that occurs during the transition from health to disease has been well documented (132, 206). Concurrently, it has also become apparent that these changes or differences in oral microbial compositions correlate with different innate responses (41). What is not well understood is the effect these different microbial populations have on inflammatory processes, especially those that have been "established" by the host to maintain health (184). Any disruption of this "established" state, whether by commensal bacteria, pathogenic bacteria or a compromise in the local or systemic health of the host will lead to an altered host condition, resulting in disease. With this in mind, one begins to appreciate the critical importance of host regulation within the sentinel state of the innate host defense system, and its relation to the pathogenesis of periodontal disease(s).

Although once thought of as the nonspecific arm of the inflammatory response, molecular advances have revealed that, in fact, a unique specificity does exist within the innate immune system. This specificity occurs due a consensus within the repertoire of receptors and their specific ligands that have evolved to allow for rapid identification and response to nonself (96) or to danger (143). For example, structural constraints within the binding capacity of pattern recognition receptors represent a way that the host uses molecular recognition to distinguish between self-carbohydrate structures and microbial carbohydrate components. In addition, lipopolysaccharide binding protein–CD14-dependent presentation of microbial components also helps define the molecular interaction during the initial interaction between microbial components and lipopolysaccharide binding protein, or during transfer of this lipopolysaccharide binding protein–microbial component to CD14, and during subsequent interactions with membrane bound cell activation complexes on the host cell itself. These interactions contribute to the fact that inflammatory mediators, made in response to gram-positive bacteria, have significantly less activity than comparable amounts of gram-negative lipopolysaccharide during *in vitro* and *in vivo* inflammatory assays as well as differential responses to different gram-negative lipopolysaccharides themselves (11, 42, 177, 196). In addition, specific structural differences, such as fatty acid acylation and phosphorylation, are key components in host cell responses to bacterial inflammatory mediators. Another advance in understanding the role of specificity within the innate immune system has come from the discovery that different toll-like receptors recognize different molecular components, thereby activating different innate pathways. To accomplish this, Toll pattern recognition receptors work in conjunction with serum soluble proteins, other Toll receptors or membrane bound proteins, thus enabling host cells to differentially identify and respond to gram-positive or gram-negative bacteria. These interactions, in turn, enable the host cells to sample and sort through their current environmental condition, in terms of commensal or pathogenic bacterial presentation, and selectively mount an appropriate response.

Advances in molecular characterization of innate response have revealed that immunoregulation of the innate response may occur at several different locations within the periodontium, or by several different mechanisms, none of which is mutually exclusive. Both gram-positive and gram-negative

bacteria are constantly shedding specific inflammatory mediators into the periodontal tissues and the host has evolved specific mechanisms to identify these released mediators. This constant flow of inflammatory products creates a ''molecular dialogue'' between the bacteria and the host that differs in health and disease. Therefore, to establish health, a basal or constitutive expression of innate effector molecules and receptors is present and facilitates molecular recognition through dialogue with bacteria and bacterial products within the gingival tissues. In addition, this dialogue results in host receptor expression levels changes facilitating a form of innate immunoregulation that ensures continued appropriate effector responses. Disruption of any of these factors can and does lead to disease (38). Therefore, the ''status'' of the host, whether it be health or disease, is established through the interplay of bacteria and host tissues, involving both molecular recognition and immunoregulation within the innate immune system.

The innate host defense status is different in health and disease

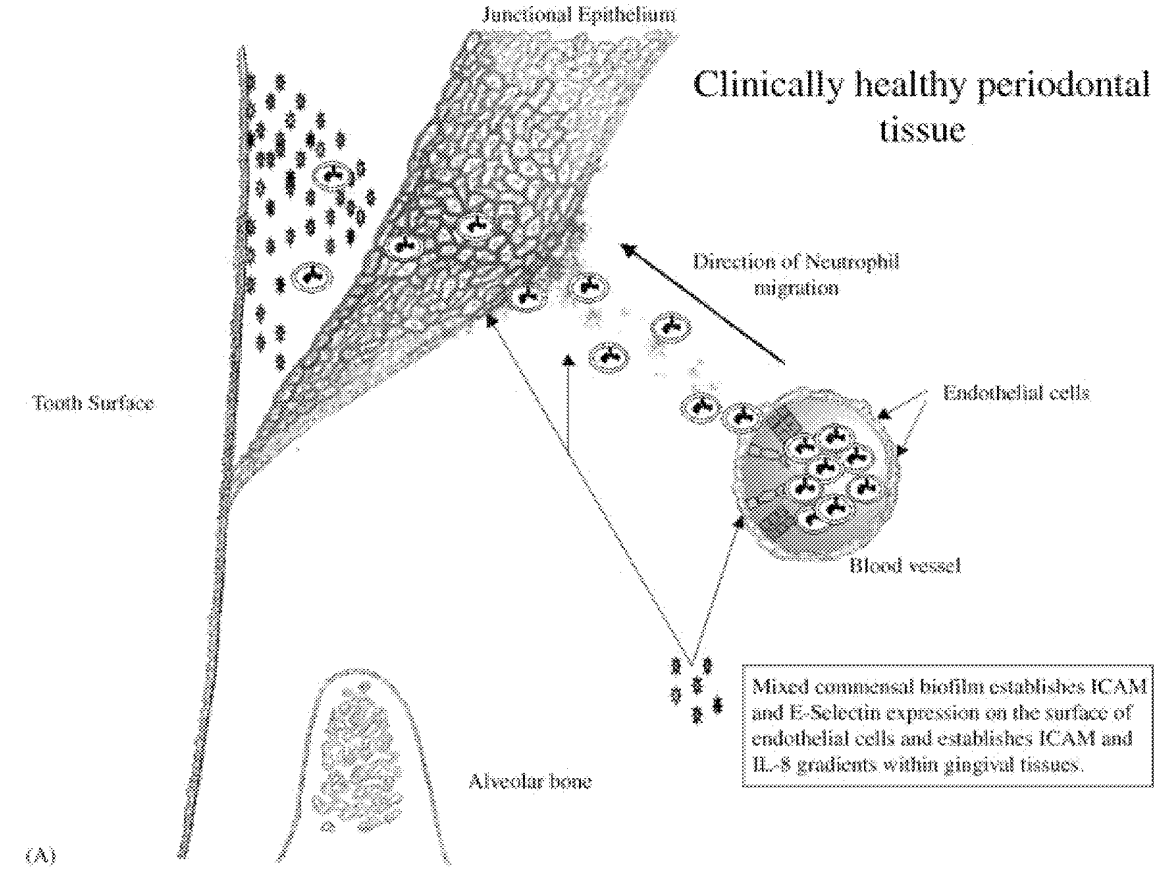
Health

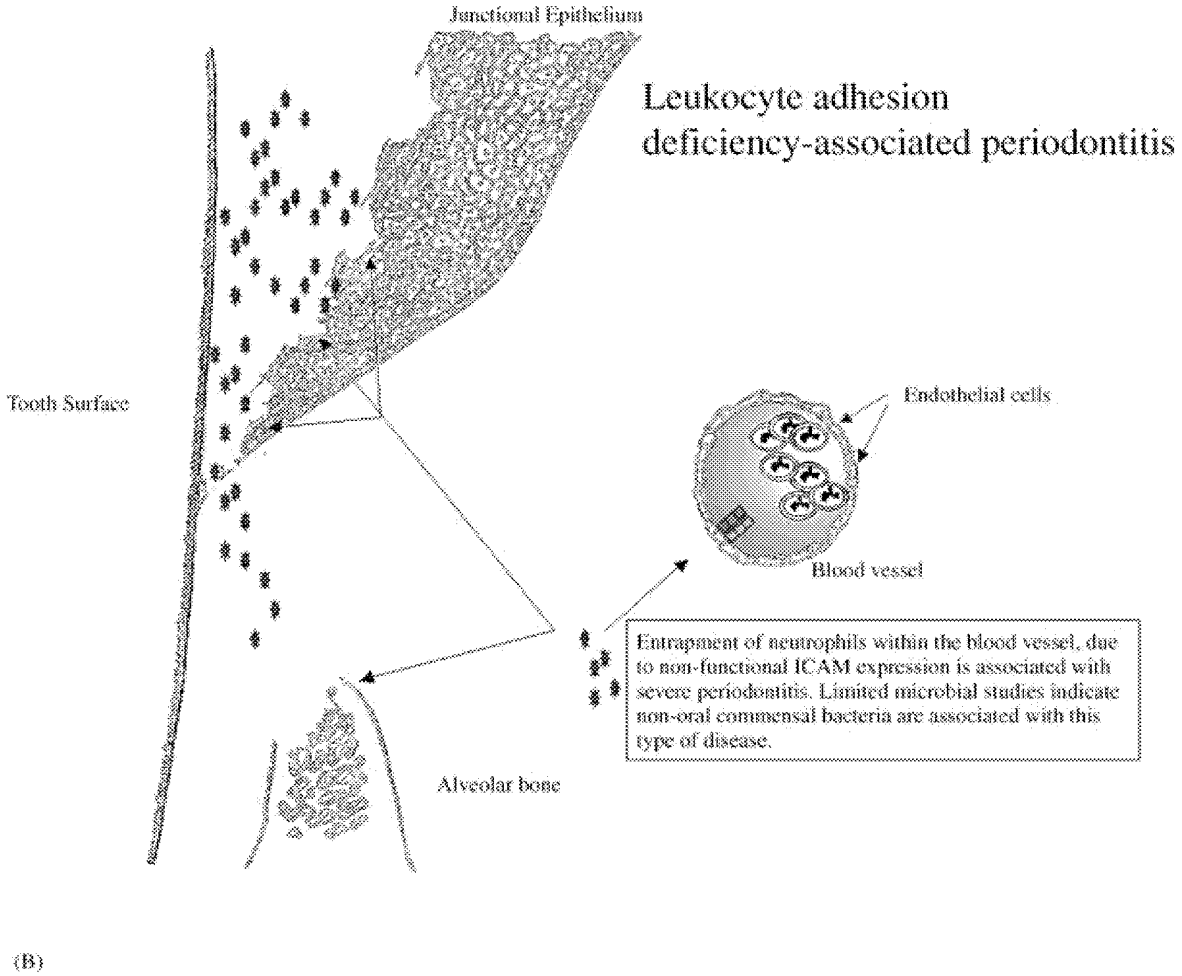
The establishment of health within the gingival tissue results from an ongoing and active event comprising the institution of a ''wall'' of neutrophils strategically located between the bacteria and the junctional epithelium. Neutrophil infiltration occurs within the periodontal tissues closest to the colonized tooth root surface (117). Immunohistochemical and *in situ* analysis suggests involvement of low level E-selectin and intracellular adhesion molecule 1 (ICAM-1) expression, which likely facilitates extravasation of the neutrophil out of the vasculature and accumulation in the gingival tissue (165) (Fig. 1A). Expression of E-selectin on endothelial cells creates a tethering interaction between the leukocyte and the endothelial cell wall, initiating a ''rolling'' stage necessary for leukocyte exit (195). Once in the tissue, it is postulated that the neutrophils ''crawl'' along cells using the ICAM receptor and follow a concentration gradient of interleukin (IL)-8, a potent neutrophil chemotactic cytokine that has been established by the resident epithelial, fibroblasts or macrophages responding to the bacteria (151, 211). Interestingly, this E-selectin and ICAM gradient, within the vasculature and junctional epithelium, is established well before any overt clinical or histologic signs of inflammation become apparent (151, 157, 165). The expression of these host mediators serves as a means by which the innate sentinel state is established and is therefore an intricate component of clinically healthy periodontal tissues. Other chemotactic factors and their host receptors, such as the f-Met-Leu-Phe (fMLP) receptor, may also contribute to the healthy state of periodontal tissues; however, their direct mechanisms are not yet fully understood (166, 216).

Disease

Loss of a functional inflammatory infiltrate leads to significant alterations in the health of the periodontal tissues. *In situ* hybridization using 35S-labeled riboprobes in frozen sections of bacterially infected and inflamed periodontal tissues revealed maximal IL-8 expression and neutrophil accumulation within the junctional epithelium adjacent to the infecting microorganisms (210). In the same study, MCP-1, a potent macrophage chemotactic cytokine, showed increased expression, along with a corresponding macrophage accumulation along the basal layer of the oral epithelium. In a related study, monoclonal antibodies for E-selectin (ELAM-1) and ICAM-1 revealed a gradient expression that was highest within connective tissue adjacent to the junctional epithelium in experimentally induced gingivitis (151). Alteration of neutrophil/macrophage diapedesis, chemotaxis and migration leading to an absence

Fig. 1. Microbial contributions to the innate host defense status of healthy and diseased periodontal tissue. Healthy periodontal tissue is protected from infection by the continuous transit of neutrophils from the highly vascularized tissue surrounding the tooth root surface into the gingival crevice. (A) One molecular mechanism by which the specific microbial consortium associated with healthy periodontal tissue facilitates this neutrophil transit. Defects within the innate host defense system (B) and specific periodontal pathogenic bacteria (C) can disrupt this key protective feature of healthy tissue and result in disease. (B) Current knowledge about the innate host defense status in individuals with leukocyte adhesion deficiency (LAD, a congenital disease that results in the formation of a non-functional intracellular adhesion molecule (ICAM) receptor). Individuals with this defect in innate host defense display a severe form of periodontitis that does not require specific periodontal pathogens. (C) The authors' hypothesis (supported by *in vitro* observations) that *P. gingivalis* creates an innate host defense defect that also disrupts neutrophil transit through the periodontium. The mechanisms by which this disruption of normal periodontal tissue function results in disease are currently not understood.


Junctional Epithelium
Clinically healthy periodontal tissue
Direction of Neutrophil migration
Tooth Surface
Endothelial cells
Blood vessel
Mixed commensal biofilm establishes ICAM and E-Selectin expression on the surface of endothelial cells and establishes ICAM and IL-8 gradients within gingival tissues.
Alveolar bone
(A)


Junctional Epithelium
Leukocyte adhesion deficiency-associated periodontitis
Tooth Surface
Endothelial cells
Blood vessel
Entrapment of neutrophils within the blood vessel, due to non-functional ICAM expression is associated with severe periodontitis. Limited microbial studies indicate non-oral commensal bacteria are associated with this type of disease.
Alveolar bone
(B)

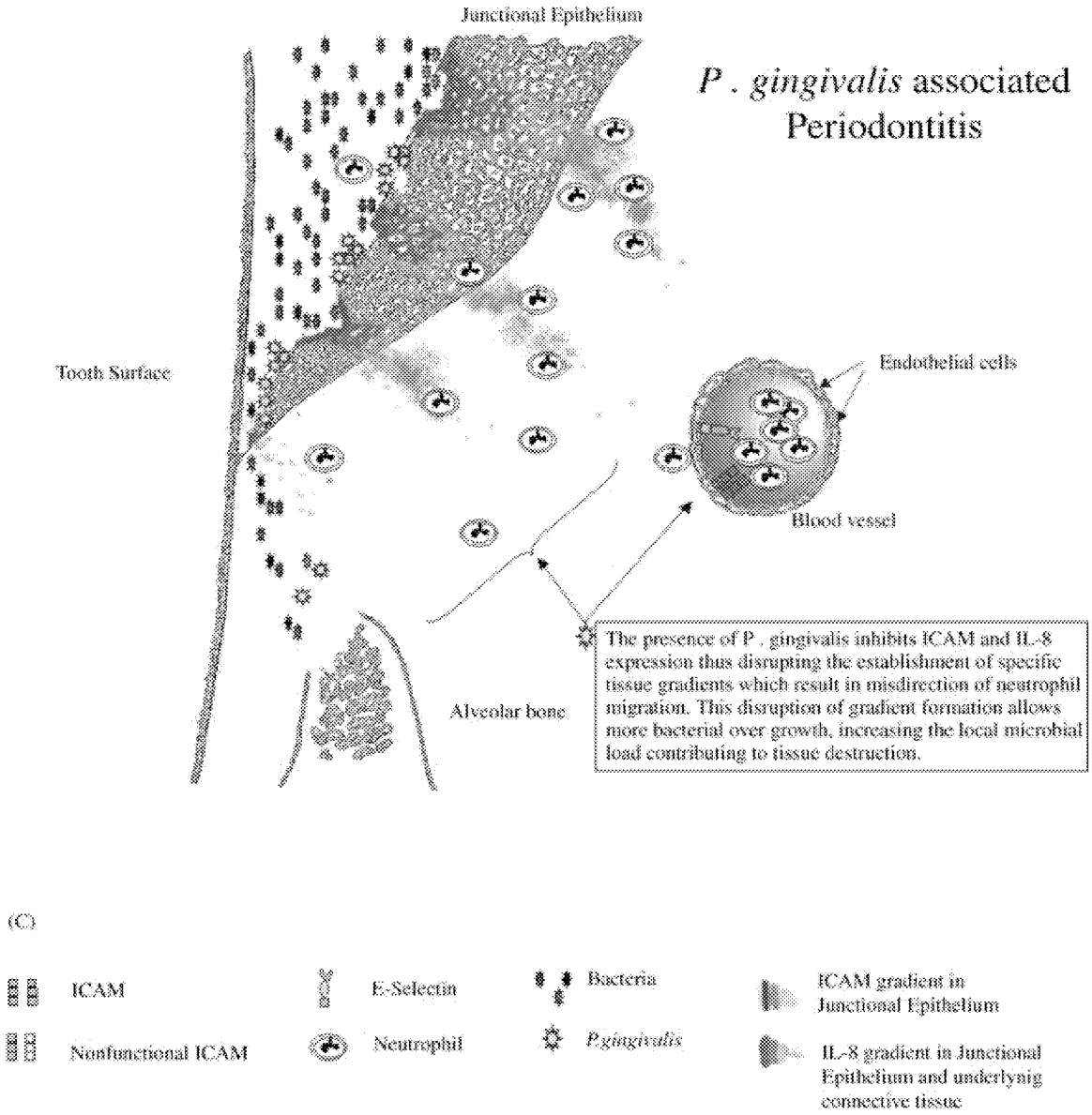


Fig. 1. continued

of the protective inflammatory barrier, or defects within the neutrophil or macrophage themselves render the host susceptible to a wide variety of bacterially induced disease. These innate cellular abnormalities can occur due to congenital defects or deficiencies of the host (31, 78, 222) or by immunosuppressive agents used to treat other systemic diseases (9, 80, 185, 233), environmental and behavioral factors (58, 65, 66) or a variety of strategies designed by the pathogen itself to avoid the protective mechanisms of the innate system. Regardless of the cause, evidence suggests that during the innate host response phase, diminished or altered function, and/or localization of neutrophils or macrophages, is critical for the establishment and severity of chronic inflammatory diseases (41).

Microbial compositional shifts

The transition from health to disease in the periodontal tissues is accompanied by a change of the plaque microflora from predominantly gram-positive aerobic cocci to predominantly gram-negative rods (133). Although hundreds of species of bacteria have been identified in plaque, the total bacterial load which can be cultured from an individual healthy sulci is relatively low. As reported in health, the dental plaque biofilm consists mostly of gram-positive species of bacteria including actinomyces and streptococci, with approximately 15% gram-negative species being found (206). In contrast, periodontally diseased sites show a corresponding increase in the number of gram-negative organisms (15–50%) (206).

Specific bacteria, such as *Fusobacterium nucleatum* have been implicated in assisting this transition from health to disease not only due to their high numbers in gingivitis and periodontitis, but also by facilitating co-aggregation between gram-positive and gram-negative bacteria (149, 226).

Accompanying this compositional microbial transition from health to disease is an increase in total bacterial numbers. Approximately 10^2–10^3 bacteria are found in healthy periodontal tissue (206), increasing to 10^4–10^6 organisms during gingivitis (206) and escalating as high as 10^5–10^8 organisms during periodontitis (41). It is thought that disease ensues when this elevation in bacterial count crosses a homeostatic threshold (158). Although bacteria can be found as free floating isolates, much of the current emphasis is being placed on bacterial biofilm formation. Dental plaque oral biofilms have been defined as ''matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interphases'' (33). In short, bacteria bind to a pellicle formed from saliva and crevicular fluid and to one another by specific and nonspecific interactions in a highly specific succession of species, thereby forming a biofilm. These adhesive interactions within the biofilm have been shown to be very stable and resistant to removal. In addition, perhaps one of the most significant consequences of recalcitrant biofilm formation is the provision of a constant source of microbial antigens to periodontal tissues. Therefore, clinical treatment is usually based upon removal or reduction of the bacteria through mechanical measures or the use of selective bacteriostatic or bacteriocidal antibiotics locally or systemically, which is consistent with other medical treatment models dealing with bacterial infections (41, 126, 193).

Structures released from bacteria enter the tissue and act as molecular modulins

Microbial composition characteristics and increased bacterial numbers undoubtedly affect the amount and type of bacterial antigens that are released into periodontal tissues. Within the dental biofilm, soluble components of the plaque organisms as well as membrane vesicles are shed into the crevicular fluid and permeate the gingival tissues (63, 156, 188, 227). These shed components modulate the inflammatory response in the local tissues by interacting with specific receptors on the various cell types in the gingival tissues, such as CD14, integrins, lipopolysaccharide binding protein, moesin, scavenger receptors, toll-like receptors, MD-2 and MyD88, (3, 6, 41, 81, 145, 189, 205), and inducing the expression of inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukins and prostaglandins. This, in turn, initiates an orchestrated cascade of activation that includes cells of myeloid and nonmyeloid origin as well as an adaptive immune response via direct and indirect pathways (41).

The most extensively studied bacterial modulator of immune/inflammatory response has been lipopolysaccharide. Lipopolysaccharide is a key component of the cell wall of gram-negative bacteria. It is composed primarily of sugars, fatty acids and phosphate. Lipopolysaccharide consists of three regions: a phosphorylated glucosamine disaccharide substituted with fatty acids known as lipid A, a highly variable O-polysaccharide, and a conserved core oligosaccharide that links the lipid A to the O-polysaccharide. Of these three regions, it is the lipid A that possesses the majority of the activity inducing an inflammatory response (Table 1). Lipopolysaccharide from oral bacteria have the same general lipid A structure (better known as the enterobacterial type), consisting of a β-(1,6)-glucosamine disaccharide substituted with hydroxylated and nonhydroxylated fatty acids in addition to phosphate (150). Complete lipid A structures are known for only a few oral species, notably *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* (123, 142), but it is clear that some are similar to *Escherichia coli* and others are significantly different (Table 1). *A. actinomycetemcomitans*, an organism associated with localized juvenile periodontitis, has a lipid A structure differing from that of *E. coli* only in the replacement of the dodecanoate with tetradecanoate on the 2' linked hydroxy-tetradecanoic acid. This results in a structure identical to that reported previously for *Haemophilis influenzae* (142). Studies of the isolated lipid A of *P. gingivalis* lipopolysaccharide reveal a structure similar to the lipid A of *Bacteroides fragilis* (225) with a small number of long chain fatty acids linked to a β-(1,6)-D-glucosamine disaccharide, phosphorylated only at the 1-carbon position (Table 1).

Other modulators of inflammation derived from periodontal bacteria are much less studied. Although gram-positive bacteria do not contain lipopolysaccharide they do contain a phosphorylated glycolipid with some structural similarities to lipopolysaccharide known as lipoteichoic acid. Lipoteichoic acids from a variety of bacteria including oral gram-positive bacteria have been reported to activate host cells, although at a much lower potency than lipopolysaccharide (22, 107, 199). Peptidoglycan is a component

Table 1. Lipid A and core composition and select biologic activity of lipopolysaccharide from various oral bacteria

Organism	3-Hydroxy fatty acids									
	Heptose	KDO	#P/LA	Short	Medium	Long	Other	Shwartzman	E-selectin	Ref.
E. coli[*]	+	+	2	–	H14	–	–	+	+	(156)
A. actinomycetemcomitans	+	+	2	–	H14	–	DD-Hep	+	+	(141, 142, 170, 171)
C. rectus	+	+	2	–	H14, H16	–	–	+		(122)
F. nucleatum	+	+	2	–	H14, H16	–	–	+		(141, 163)
H. parainfluenzae	+	+	2	–	H14	–	–	+		(213)
L. buccalis	+			–	–	–	–	+		(114)
B. fragilis[*]		+	1	–	–	H17i	–	–	–	(202, 225)
P. gingivalis	+/–	+	1	–	–	H17i	P-KDO	–	–	(24, 55, 101, 141, 159, 187)
T. forsythia				–	–	H17i	–		–	(61)
B. loescheii				–	–	H17i, H16	–	–		(101, 141)
P. intermedia	+/–	+		–	–	H17i	P-KDO	–	–	(100, 101, 141)
C. sputigena	+	+			–	H17i, H15i	–	–		(43, 174)
S. sputigena	+	+		H13	–	–	GalN in LA	+		(124)
E. corrodens				H12	–	–	–	+		(141)
C. periodontii	+	+		H13	–	–	–	+		(115)
Vellonella sp.	+	+		H13	–	–	–	+		(23)
T. denticola				H12, H13	–	–	–			(37)

[*]Non-oral species.
DD-Hep = D-glycero-D-mannoheptose. P-KDO = phosphorylated KDO.
+ Component has been reported. – absence of component has been reported. Blank boxes indicate the component or activity has not been reported. Adapted from Ref (11).

of the cell wall of both gram-positive and gram-negative bacteria and also stimulates inflammatory mediator production, again at a lower potency than lipopolysaccharide. Peptidoglycan, another identified inflammatory molecule, has been isolated from several periodontal organisms (19) and stimulates prostaglandin production in human monocytes when applied in the microgram per milliliter range (18). Other factors such as purified fimbriae or synthetic peptides from *P. gingivalis* have been shown to activate human gingival epithelial cells to secrete IL-8 (191). Henderson (18) has reported that lipid A binding proteins from *P. gingivalis* are able to stimulate IL-6 secretion in human gingival fibroblasts. This last observation has practical significance with regards to evaluating the activity of lipopolysaccharide preparations since it indicates that part of the activity ascribed to lipopolysaccharide preparations may sometimes be due to tightly bound lipid A associated proteins that are present as contaminants. A non-endotoxic glycoprotein isolated from *Prevotella intermedia* was found to induce a high level of IL-8 activity in human monocytes, although the chemical identity of this material did not appear to be highly defined (54). An important finding in terms of host inflammatory modulation comes from research involving *P. gingivalis*. Cysteine proteases from *P. gingivalis* have been shown not only to stimulate the release of inflammatory mediators from cultured gingival cells but also to have the ability to actively degrade cytokines (33, 67). This degradation of host mediators is another tool that bacteria may use to modulate the inflammatory condition to their benefit and is likely to be important in the development and progression of periodontitis. Besides lipopolysaccharides and lipoteichoic acid, the host may respond to other bacterial products, including lipoproteins, fimbriae, porins, Lipid A associated proteins, peptidoglycans, heat shock proteins and nucleic acids (25, 56, 71, 82, 137, 191, 200).

In summary, the innate immune system has developed highly structured and specific effector mechanisms to respond to bacterial products that are known to permeate the gingival tissue. These mechanisms give an insight into the complex interaction of commensal bacteria, pathogenic bacteria and the appropriate host response and are essential for the system designed for the immunosurveillance of the periodontium. In addition, there is convincing evidence that these innate effector mechanisms and their interaction with specific bacterial inflammatory modulins evolved prior to the emergence of adaptive immunity (96, 144).

Innate defense specificity occurs via a complex network of host receptors and signaling molecules

Pattern recognition receptors

Dental plaque bacteria interact with host cells either by direct adherence mechanisms, invasion of specific cells such as gingival epithelial cells or the production of components that are recognized directly by the host immune cells via *pattern recognition receptors*. These receptors identify specific structural patterns with the appropriate spatial orientation that are unique to bacterial cells (172, 212). For example, lipopolysaccharide, double-stranded RNA, unmethylated CpG motifs, specific mannans and glycans are all examples of microbial components that contain essential structural features that cannot be modified by the organism without changing its core structure (96, 144). Although variability and molecular heterogeneity exists within bacterial components, alteration of *these* essential structural features would destroy pathogenicity altogether or alter some indispensable bacterial function that is essential for its survival (144).

Pattern recognition of conserved core bacterial components serves as the basis for specificity within innate host cells and innate cells have, in fact, *evolved* to recognize a wide range of distinct microbial patterns (96). By recognizing common bacterial patterns rather than the milieu of different bacterial structures, polyspecificity is ensured within the innate immune system. This polyspecificity enables a rapid response by a relatively small number of host effector cells armed with a constitutive repertoire of receptors, identifying a range of distinct but conserved microbial pattern(s). In addition, this recognition and response occurs without the need for clonal expansion of specialized adaptive cells with highly specialized receptors for specific foreign structures (96). Furthermore, pattern recognition receptors have the ability to discriminate between self, nonself structures and danger signals due to the structural heterogeneity that exists within the microbial component recognized (143). This discrimination assists in effectively identifying commensal or pathogenic species within the local tissue environment and/or initiating the adaptive response when challenged with specific or unique pathogenic bacteria that cannot be effectively eliminated.

Lipopolysaccharide binding protein/ CD14

Lipopolysaccharide has been shown to activate a variety of cell types in CD14-dependent manner. Lipopolysaccharide binds to the serum protein lipopolysaccharide binding protein and is transferred to either soluble CD14 (sCD14) or membrane bound CD14 (mCD14). Some cell types (monocytes, neutrophils) have mCD14 present on their cell surface as a glucosylphosphatidylinositol anchored protein, whereas others (endothelial cells) rely on the presence of soluble protein. The CD14-bound lipopolysaccharide interacts with a receptor complex on the cell surface that includes toll-like receptor 4 and the accessory protein MD-2 and initiates one or more intracellular pathways leading to expression of inflammatory mediators. Binding of lipopolysaccharide to CD14 has been shown to be the result of electrostatic interactions with numerous charged amino acids present on CD14. It is likely that the nature of this interaction allows CD14 to act as "molecular flypaper" (118), binding not only structurally diverse lipopolysaccharide but also other bacterial components such as peptidoglycan and lipoteichoic acid (199, 214). Although CD14 binds a variety of different microbial components, studies have demonstrated that different host responses to specific microbial structures occur after CD14 binding (44, 111).

Specific microbial component recognition via toll-like receptors

Toll-like receptors have been shown to recognize different microbial components (6, 147). These receptors were named in reference to the class of genes in *Drosophila* known as *Toll*. *Drosophila* Toll was originally noticed for its role as a developmental gene. Later it was found that Toll was part of the innate immune response of *Drosophila* to bacterial and fungal pathogens. The involvement of related

proteins (toll-like receptors) in the mammalian response to pathogens was discovered later from two separate lines of research. One line of research that led to Toll was the discovery that the gene (*Lps*) responsible for lipopolysaccharide unresponsiveness in mouse non-responder strains, particularly C3H/HeJ and C57BL/10ScCr, mapped to the locus of toll-like receptor 4 (6, 145, 147, 178). Independent of this line of work, Yang et al. (231) and Kirschning et al. (110) employed transfection-based strategies to identify toll-like receptor 4 as the lipopolysaccharide transducer among the family of toll-like receptor proteins. Subsequent work has indicated that other toll-like receptors are important in the host response to other bacterial ligands. Toll-like receptor 2 has been implicated in response to lipoteichoic acid (164, 201), lipoproteins (25, 56), fimbriae (70) and peptidoglycan (82, 145); toll-like receptor 3 in response to double-stranded RNA (4) and toll-like receptor 9 in response to DNA (81). Toll-like receptor 1 and toll-like receptor 6 have been reported to act synergistically with toll-like receptor 2, apparently by forming heterodimers (71). Other toll-like receptors remain largely uncharacterized with regard to function.

Various molecules are known to form complexes with toll-like receptors at the host cell membrane. These include CD14, the secreted factor MD-2 and the adaptor protein MyD88, although an MyD88 independent pathway has been described (3, 189, 191, 205, 224). Additional molecules continue to become known, including Mal and Tollip (91, 145). In the case of lipopolysaccharide, the ligand is complexed to CD14 and then forms a complex at the cell surface that includes toll-like receptor 4 and MD-2 (3, 220). Signal transduction includes recruitment of MyD88 and IL-1 receptor associated kinase and results in nuclear translocation and gene activation of specific inflammatory mediators including IL-1 and TNF-α (6).

Intracellular signaling within the innate immune system

Once the innate cell receptor has bound its specific ligand the response kinetics to this interaction must be immediate, thus defining one of the hallmark characteristic features of the innate immune system. To facilitate this response, specific intracellular signaling pathways have been shown to be utilized by specialized cells in their rapid response to environmental stresses, osmotic changes or inflammatory cytokines.

Mitogen activated protein kinase (MAPK) signal transduction pathways have been shown to be the most prevalent intracellular signaling pathways utilized by host cells (125). The highly conserved MAPK pathway consists of a sequential, trimolecular cascade of MAPK, MAPK kinase and MAPKK kinase (69). Upon phosphorylation, this kinase cascade activates, either separately or in combination, specific transcription factors, which then translocate into the nucleus, inducing new gene expression (186).

Effector intracellular pathways within cells of the innate immune system are activated by specific receptor–ligand interactions (Fig. 2). For example, lipopolysaccharide initiated receptor–ligand binding results in rapid tyrosine phosphorylation of upstream cytoplasmic proteins for MAPK, which have been identified as members of the *src* family of tyrosine kinases (67). Specifically, *lyn, hck* and *fgr* have all been shown to be activated in response to lipopolysaccharide stimulation in macrophages (83, 197, 221). These *Src*-family kinases seem to play an important role in the early stages of initial intracellular signal propagation and signal amplification (128), thus helping to inform the cell not only of its receptor–ligand binding activity, but also of the surrounding environmental milieu. If a kinase-associated receptor is activated, this initiates a multistep process that phosphorylates and activates multiple cytoplasmic molecules, which can ultimately lead to specific gene expression (1, 59, 128). Inhibition of protein tyrosine kinase, with the specific inhibitors herbimycin and genistein, has been shown to block 12–16-fold increases in lipopolysaccharide induced mRNA levels of IL-1β, IL-6 and TNF-α (59). *Src*-family kinases have also been shown to possess the ability to inhibit cell activation as well as be positive regulators (128). For example, selectively expressed SHPS-1/SIRPα, an ITIM containing receptor on macrophages, have been shown to be negatively regulated by the Src-family kinase Fgr in regard to FcγR-mediated phagocytosis (64).

Other intermediate signaling mediators of the MAPK associated pathways downstream to the proximal Src-family kinases, such as the small proteins transducein and Gi, have also been shown to be activated during lipopolysaccharide stimulation (95). In addition to these, activation of protein kinase C plus associated isoforms, as well as phosphatidylinositol (PI)3-kinase, have also been shown to occur (85, 86, 134, 186, 189). Inhibition of MEK via specific inhibitor U0126 has been shown to inhibit this MAPK kinase in monocytes, reducing the levels of inflammatory cytokines such as IL-1, IL-8, TNF-α,

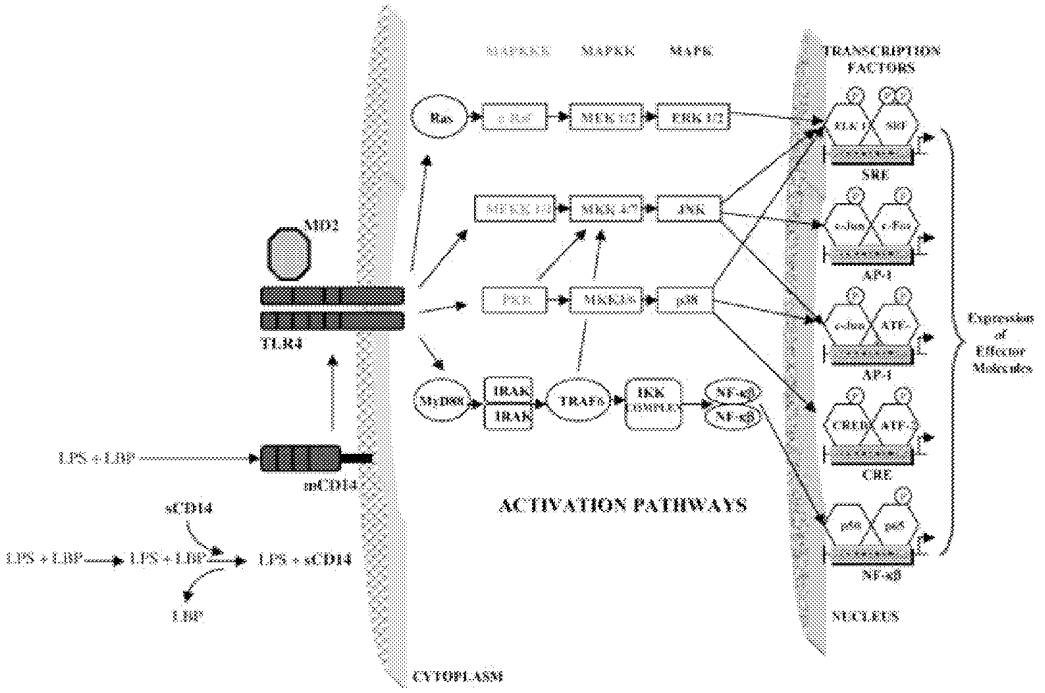


Fig. 2. Activation of specific intracellular pathways within the innate immune system leads to expression of specific effector molecules. Adapted from Guha & Mackman (67). LPS, lipopolysaccharide. LBP, lipopolysaccharide binding protein.

and prostaglandin E_2 following lipopolysaccharide activation, verifying the importance of the MAPK associated pathway (s) in the host response to noxious stimuli (68, 186).

Extracellular signal-related kinases (ERK 1/2) (26, 134), c-Jun amino terminal kinases (JNKs) (72), and p38 (32) have all been elucidated as specific downstream members of the MAPK family of protein kinases (68). These distinct and sometimes overlapping pathways are located distal to the protein tyrosine kinases involved in the initiation and amplification of the intracellular signal. These downstream pathways aid the cellular mechanisms that help regulate changes in gene expression after specific receptor ligand recognition at the cell surface (69).

It has been shown that in cells of myeloid origin, the extracellular signal-related kinase (ERK 1/2) is activated during lipopolysaccharide stimulation (47, 67, 139) but the exact mechanism is not clearly understood. Through a TEY motif, the kinase domains of ERK 1/2 become activated via phosphorylation (67). This is thought to occur in a Raf-1 dependent manner (183) and inhibition of the lipopolysaccharide induced TNF-α promoter has been shown for both Ras and c-Raf (60), supporting the importance of the Ras → c-Raf (MAPKKK) → Mek 1/2 (MAPKK) → ERK 1/2 (MAPK) pathway in the expression of TNF-α after lipopolysaccharide stimulation (67). However, lipopolysaccharide activation of the MEK-ERK 1/2 pathway independent of the c-Ras pathway has also been identified (30), indicating that a common feature of eukaryotic intracellular signaling is, perhaps, multiple activation pathways and/or multiple means of single pathway activation. The c-Jun N-terminal kinase pathway has been identified as another significant member of the MAPK pathway family. In a manner similar to the ERK 1/2 TEY motif, JNK kinases have TPY motifs within their kinase domains and are activated upon phosphorylation (67). This activation has been shown to occur during lipopolysaccharide stimulation of both murine monocytes (RAW 264.7) and human monocyte (THP-1) cell lines (72). Upstream MAP kinases of the JNK pathways include MEKK 1/4 (MAPKKK) and MKK 4/7 (MAPKK) and have also been shown to become activated in response to multiple inflammatory stimuli (67). Inhibition of the JNK pathway through specific c-Jun N-terminal kinase inhibitors not only interferes with inflammatory stimulus signals but also has been shown to block transcription of specialized proteins, cellular apoptosis and metabolism in specific cells (77, 203).

An additional pathway of the MAPK pathways consists of p38 MAP kinase. p38 has been described as a 38-kDa polypeptide that becomes phosphorylated in response to endotoxin treatment and osmotic shock (77). Consistent with the previous MAPK pathways, the p38 pathway also contains TGY motifs in its kinase

domain (53, 67). Both the ERK and p38 pathways are considered activated when the cell is under environmental stress and/or inflammatory cytokines. These pathways have been shown to be very important in the inflammatory response and have been referred to as "stress-activated kinases" (125). Inhibition of the p38 pathway prior to lipopolysaccharide stimulation decreased IL-1α, IL-1ra and TNF-α production, also supporting the importance of this pathway in inflammatory signaling (179). Upstream signaling molecules within this pathway that are involved in p38 activation include Cdc42, PAK and Rac1, (10, 207, 236) and multiple MAPKKs involved include MKK3, MKK6 and MKK4 (45, 67, 76, 180).

Activation of the MAP kinase cascade(s) initiates activation of multiple downstream transcription factors via phosphorylation. For example, one of the downstream targets of the MEK-ERK 1/2 pathway is the transcription factor Elk-1; if phosphorylated, the C-terminal domain induces a conformational change that results in an inducible complex including Elk1 and SRF (67). In the JNK pathways, downstream transcriptional factors include ATF-2, Elk-1 and c-Jun (104), which have been discovered to be important transcription factors involved with encoding inflammatory mediators (67). One important role of the p38 pathway is in the translation of cytokine mRNA and cytokine production (129). Through the use of p38 inhibitors (i.e. SB203580), activation through the p38 pathway and expression of IL-1 and TNF-α was reduced following lipopolysaccharide stimulation, confirming the role of protein phosphorylation in the response of monocytes and macrophages to endotoxin (67, 129). Additional inhibition studies, such as transmigration studies in monocytes, have demonstrated two distinct signaling cascades, leading to ERK induced integrin activation and p38 induced chemotaxis via MCP-1 (8).

Upstream of the IKK-NF-κβ pathway, MyD88, IL-1 receptor associated kinase and TRAF6 have been shown to play a role in lipopolysaccharide signaling in monocytes and macrophages (67, 131). In specific, MyD88 and TRAF6-deficient mice were either hyporesponsive or lacked the ability to respond to lipopolysaccharide stimulation (105, 135). Through a TIR domain, (154, 155) the MyD88 adaptor protein interacts with a TIR domain on Toll or IL-1R. The other end of MyD88 interacts with the serine/threonine innate immunity kinase IL-1 receptor associated kinase, whch then initiates phosphorylation of an inhibitory protein Iκβ that holds the transcription factor, NF-κβ in the cytosol (48, 94, 162). NF-κβ translocates into the nucleus once Iκβ is phosphorylated after cellular stimulation and then activates genes involved with inflammatory cytokine production and immunity (94).

Bacterial clearance

Binding to receptor molecules does not necessarily lead to an inflammatory cytokine expression and, in fact, may more frequently lead to clearance of the bacterial component. For example, recent reports indicate that lipopolysaccharide binding protein, best known for its ability to enhance CD14-dependent activation of host cells by lipopolysaccharide, promotes phagocytosis of *E. coli* by macrophages (112) and enhances the clearance of lipopolysaccharide from the blood (237). Similarly, bactericidal/permeability-increasing protein, a structurally related protein that does not enhance the inflammatory response to lipopolysaccharide, has been shown to inhibit the ability of lipopolysaccharide to activate neutrophils (87) and to promote phagocytosis of lipopolysaccharide by mononuclear cells (29). Soluble CD14 (sCD14) can act to neutralize lipopolysaccharide by effecting transfer to serum lipoproteins (229), which have been shown to effectively neutralize lipopolysaccharide in both *in vitro* and *in vivo* experimental systems (51). Uptake of lipopolysaccharide by monocytes is also known to involve CD14 and whether the interaction leads to activation or neutralizing clearance may depend on the aggregation state of the lipopolysaccharide (57, 217). Scavenger receptors are expressed by macrophages, Kupffer cells and some endothelial cells (74, 169). These receptors bind bacteria and bacterial products such as lipopolysaccharide, lipoteichoic acid and CpG DNA, and promote their uptake and clearance (74, 169). Mannose binding protein has been reported to bind mannose rich bacteria and opsonize them for clearance by phagocytes (121). There is also evidence that opsonization by natural anti-lipopolysaccharide antibodies can lead to enhanced clearance (182), presumably by an Fc-mediated process (28).

Immunoregulation within the innate immune system

Although cells of the innate host defense recognize periodontal bacteria, there are differences in the way they recognize the bacteria and the type and degree of response that follows. Differences in the way the host cells use receptors to interact with a particular

bacterium explain in part the observed variation in the response to the organism or its components. For example, it is known that the response to some bacteria is enhanced by the serum protein lipopolysaccharide binding protein (176, 208, 209). But while lipopolysaccharide binding protein is a strong enhancer of the response to lipopolysaccharide and other lipid containing products, it has a variable effect in terms of response within the same cells to bacterial components such as peptidoglycan, fimbriae or lipoteichoic acid (7, 49, 84). In addition, lipopolysaccharides with reduced amounts of lipid moieties cannot utilize lipopolysaccharide binding protein to transfer to CD14 (12). Binding to CD14, on the other hand, appears to depend significantly on the charge of the bacterial ligand (36). CD14 is found both in serum and as a host cell membrane component present on the surface of some cell types such as monocytes and neutrophils but not other cell types such as endothelial cells (176, 208). The ability of a cell type to utilize membrane CD14 versus serum CD14 may determine whether the cell can respond to particular bacterial products. Lipopolysaccharide from *E. coli* can activate IL-8 secretion from both mCD14 containing monocytes and endothelial cells in the presence of serum, indicating an ability to utilize either form of CD14. Lipopolysaccharide from *P. gingivalis*, however, can stimulate secretion of IL-8 from monocytes but not endothelial cells (2, 17, 36, 161). The ability of *P. gingivalis* lipopolysaccharide to activate some cell types but not others may indicate that the host response to *P. gingivalis* lipopolysaccharide, in the gingival tissues, is disrupted due to the unbalanced response towards this unique lipopolysaccharide. In addition, *P. gingivalis* lipopolysaccharide has been shown to inhibit the ability of other bacteria to activate CD14 negative cells (40), suggesting that this lipopolysaccharide may disrupt the normal host response to *other* bacteria present in periodontal tissue.

Toll-like receptors are also known to discriminate between bacterial ligands. Although the vast majority of the work done on the specifics of toll-like receptor activation has been done in non-oral bacteria, especially *E. coli*, there is a body of work which either has been done on periodontal pathogens (primarily *P. gingivalis*) or can be extrapolated to relevant organisms. Lipopolysaccharide from *E. coli* was the first bacterial component examined in the toll-like receptor activation system and it remains by far the best studied. Lipopolysaccharide was originally believed to activate cells through a toll-like receptor 2 pathway but it was soon discovered that the main receptor complex for lipopolysaccharide includes toll-like receptor 4 (6, 46, 92, 145, 147). Initial studies in non-responder mice and cells transfected with toll-like receptor genes may have been affected by the presence of contaminating bacterial products in the lipopolysaccharide preparation. Binding and recognition of lipopolysaccharide by toll-like receptor 4 is at least somewhat species specific and the recognition of the exact lipopolysaccharide structure requires the presence of the molecule MD-2 (3, 219, 220). Cells from humans, mice, or hamsters, differentially recognize partial structures from Lipopolysaccharide such as Lipid IVA, and the difference is related to the species source of the toll-like receptor 4/MD-2 present in the cells (3). Lipopolysaccharide isolated from the periodontal bacterium *P. gingivalis* was also shown to utilize toll-like receptor 2 and not toll-like receptor 4 (12, 140). This result is reasonable since *P. gingivalis* lipopolysaccharide has a different lipid structure than *E. coli* lipopolysaccharide and may utilize receptors differently, as has been observed for lipopolysaccharide partial structures. Consistent with this idea, many reports indicate that *P. gingivalis* lipopolysaccharide induces different responses in cultured cells and in animal models. Some authors, however, have reported that *P. gingivalis* lipopolysaccharide utilizes primarily toll-like receptor 4 (204). This discrepancy may be due to the difference in cell and animal models employed or differences in contents of the lipopolysaccharide preparation with regards to heterogeneity or contamination.

Although toll-like receptor 2 was originally believed to be the primary lipopolysaccharide receptor, it is now believed that toll-like receptor 2 is involved in the recognition of a variety of other components including lipoteichoic acid, lipoprotein, fimbriae, glycoprotein and phenol soluble modulin (25, 56, 71, 103, 145, 200, 204, 231). Components from oral bacteria known to activate toll-like receptor 2 are *P. gingivalis* fimbria, Treponema lipoprotein and glycolipid and glycoprotein from *P. intermedia* (7, 164, 200). Other components that likely activate via toll-like receptor 2, such as peptidoglycan and lipoteichoic acid, have not been examined.

Toll-like receptors are known to be expressed in a number of tissues and by a variety of cell types including monocytes, endothelial cells, fibroblasts, osteoblasts and dendritic cells (6, 50, 70, 108, 223). Expression of a specific toll-like receptor depends on cell type; for example, endothelial cells express predominantly toll-like receptor 4, monocytes express both toll-like receptor 4 and toll-like receptor 2, while dendritic cells express toll-like receptor

Table 2. Differential expression within gingival innate immune cells

Innate cell type	Receptor/membrane molecule	Author
Monocyte/Macrophage	Scavenger receptor	Pearson (168)
	Collectin receptor	Malhotra (138); Holmskov (89)
	TLR1, TLR2, TLR4, TLR5	Muzio et al. (153); Muzio and Mantovani (154)
	Siglecs	Medzhitov and Janeway (146)
	Integrins (LFA-1, MAC-1)	Yusuf-Makagiansar et al. (234)
	mCD14	Muhvic et al. (152)
Neutrophil	Collectin receptor	Holmskov (89)
	TLR1, TLR2, TLR4, TLR5	Muzio et al. (153); Muzio and Mantovani (154)
	Siglecs	Medzhitov and Janeway (146)
	Integrins (LFA-1, MAC-1)	Yusuf-Makagiansar et al. (234)
	mCD14	Power et al. (175)
Fibroblast	Collectin receptor	Holmskov (89)
	TLR2, TLR4	Tabeta et al. (204)
	ICAM-1	Hayashi et al. (79)
	mCD14	Sugawara et al. (198)
Endothelial	Scavenger receptor	Yeh et al. (232); Hofnagel et al. (88)
	Collectin receptor	Xiao et al. (230); Holmskov (89)
	TLR4	Muzio and Mantovani (154)
	E-Selectin	Pietrzak et al. (173)
	P-Selectin	Krugluger et al. (120)
	ICAMs	Moughal et al. (151)
	mCD14	Jersmann et al. (98)
Epithelial	Beta-defensins-2	Krisanaprakornkit et al. (119)
	TLR2,TLR4	Wolfs et al. (228); Hornef et al. (90)
Dendritic cells	TLR1, TLR2, TLR4, TLR5	Muzio et al. (153)
	TLR3	Muzio and Mantovani (154)
	Siglecs	Medzhitov and Janeway (146)

TLR, toll-like receptor. ICAM-1, intracellular adhesion molecule 1.

3 (6, 50, 70, 145, 200) (Table 2). Toll-like receptor expression may be constitutive or can be modulated by exposure to bacterial or other factors. Cells of the gingival tissue have been shown to express toll-like receptor and in some instances the expression is modulated by bacterial factors. Gingival epithelial cells have been shown to express both toll-like receptor 2 and toll-like receptor 4 (7, 52). Both toll-like receptor 2 and toll-like receptor 4 were shown to be upregulated by exposure to live *A. actinomycetemcomitans* but not by heat-killed bacteria (52). Gingival epithelial cells responded to a variety of bacterial products including lipopolysaccharide, lipoteichoic acid, peptidoglycan and whole bacteria, suggesting that multiple Toll receptors might be present and functional (7, 52, 62). This capability to respond to varied ligands might indicate that epithelial cells, which form the primary physical barrier to microbial challenge, are broadly responsive to bacteria on the epithelial surface. Wang et al. (223) showed that gingival fibroblasts express toll-like receptor 4 and that toll-like receptor 4 expression is downregulated in response to purified lipopolysaccharide from *P. gingivalis*. It would be interesting to speculate about the role downregulation might play in modulating the host response to *P. gingivalis* during infection, but it has conversely been reported that *P. gingivalis* lipopolysaccharide causes an increase in expression of both toll-like receptor 2 and toll-like receptor 4 (Table 2).

Defects within the innate immune system alter susceptibility to and severity of periodontitis

Altered susceptibility

A functional innate immune system is essential for protection against bacterial exposure within the environment of the host. Mice lacking the adhesion molecules E-selectin and P-selectin have been generated and shown to be defective in neutrophil emigration and have increased susceptibility to infection (27). Deletion of P-selectin has also been

shown to promote alveolar bone loss in a mouse model of *P. gingivalis* induced periodontal destruction, demonstrating the importance of adhesion molecules in the host response to oral pathogens (14). In the same model, defects in T and B lymphocytes were also shown to affect bone loss (13, 15), indicating that susceptibility to bacterially induced bone loss is complex and multifactorial. Macrophages from CD14 null mice were able to respond to *E. coli* by activation of NFκ-B and c-Jun signaling pathways and production of IL-6 and TNF-α. There was no response, however, to *E. coli* lipopolysaccharide, indicating that other receptors may compensate for lack of CD14 in the response to whole bacteria (148). Examinations of mice deficient in the adapter molecule MyD88 indicate that although the response to many bacterial products is mediated through MyD88 (205), pathways independent of MyD88 also exist (106). One recent study in mice is suggestive of toll-like receptor involvement in periodontitis. Using a mouse model of bacterially induced periapical bone destruction, Hou et al. (92) examined the ability of various oral bacteria to induce bone destruction in normal or toll-like receptor 4-deficient mice. It was found that when the mice were challenged with a mixed infection of putative periodontal pathogens, observed bone loss was significantly less in two strains of toll-like receptor 4 deficient mice than in wild-type controls. Further, this decrease was correlated with reduced expression of the bone resorptive cytokines IL-1α and IL-1β as well as the proinflammatory cytokine IL-12. Other inflammatory mediators (TNF-α, interferon-γ, IL-10) were not affected. In this model, at least, it appears that toll-like receptor 4 is involved in the inflammation and destruction of periodontal bone in response to an infection of relevant bacteria. The study does seem, however, to indicate a role for toll-like receptor involvement in bone loss observed during active inflammation. In the future, it may be found useful to screen for toll-like receptor polymorphisms as a risk factor for periodontitis, as has been proposed for other hereditary determinants such as IL-1 gene heterogeneity.

Immunosuppression and systemic disease

One of the most dramatic changes in innate immune system function occurs in patients undergoing immunosuppressive therapy. Significant reduction in the number of circulating neutrophils during myelosuppressive therapy can severely compromise the host by dramatically increasing susceptibility to oral infection (38). Congenital diseases associated with an increase in periodontitis severity, such as leukocyte adhesion deficiency, Chediak–Higashi syndrome, Papillon–Lefèvre syndrome and chronic/cyclic neutropenia involve numerous leukocyte defects involving: regulation of the number of circulating neutrophils, defects within the neutrophil itself and/or on the surface of neutrophils, or through molecular events controlling neutrophil diapedesis and chemotaxis (5, 20, 38, 73, 102, 222) (Fig. 1B). Alteration in the physiologic properties of the gingival vasculature could also contribute to reduced numbers or impediment of the circulating neutrophils, which, in turn, could alter host responses to bacterial challenge. This type of vascular change within the gingival tissues has been reported in both diabetic and smoking patients (21, 54, 235). Manifestation of systemic disease with or without increased susceptibility to periodontal infection is multifactorial and it is often difficult to directly identify the pathogenic etiology of a specific disease. However, it is clear that the disruption of an intact innate immune system is detrimental to the health of the host in either a localized or a systemic manner (Table 3).

Genetic polymorphisms

Some genetic deficits are less readily identifiable due to lack of severity or serious systemic effects. These may take the form of polymorphisms in genes coding for host defense molecules. Perhaps the best-known gene polymorphism that has been implicated in susceptibility to periodontitis is IL-1 (35, 116, 167, 194). The IL-1 genotype has been associated with periodontal health in early onset periodontitis (167), periodontal maintenance populations (127) and adult periodontitis (35, 116). It has also been reported to affect the bacterial composition of plaque from periodontitis patients (194). Polymorphisms in TNF-α and IL-10 have also been examined (34, 109, 190) but no link was observed for the tested variants.

Receptors for bacterial components would seem to be a likely class of host molecules that might influence susceptibility to a bacterially induced disease such as periodontitis. Gene variants do in some cases affect the host's ability to respond to bacterial challenge. For example, a polymorphic variant of lipopolysaccharide binding protein has been shown to be associated with an increased risk

Table 3. Induced or congenital deficiencies

Condition	Phenotype	Periodontitis	Significant shift in periodontopathogenic microbiota
Immunosuppressive therapy	Significant neutropenia and loss of mucosal barrier	Acute, generalized and severe	Yes, commensal bacteria are found
Leukocyte adhesion deficiency	Loss of all leukocyte movement from vascular compartment to tissue, molecularly defined as failure to express integrin adhesions or selectin receptor	Acute, generalized and severe	Yes, commensal bacteria are found
Chediak–Higashi Syndrome	Non-functioning granulocytes due to defective lysosomal trafficking regulator gene	Acute, generalized and severe	Unknown
Chronic neutropenia	Significantly decreased levels of neutrophils brought about by unknown congenital disorder	Variable, acute and chronic types, generalized and severe	Questionable, appears to have same periodontopathogens associated with adult type disease
Papillon–Lefèvre Syndrome	Hyperkeratosis of palms and soles, probable defect in neutrophil chemotaxis, defect localized to chromosome 11q14, unknown gene function	Variable, acute and chronic types, generalized and severe	Questionable, appears to have same periodontopathogens associated with adult type disease
Diabetes	Numerous complications associated with advanced glycated end-products (AGE)	Increased incidence and severity in non-controlled patients	No, appears to contain similar periodontopathogenic species found in non-diabetic individuals
Cigarette smoking	Not clear, may have decreased vascular response in periodontium	Increase incidence and severity	No
Human Immunodeficiency Virus	Impaired cell mediated immunity	Unusual forms which can be acute, severe, and generalized	Yes, *Candidia* and *Borrelia* can be found

Adapted from Darveau (39).

for incidence and lethality of sepsis in male patients (93).

The importance of CD14 in host defense suggests that genetic variation in this receptor may have significant consequences for susceptibility to infectious diseases. Several polymorphisms in the CD14 gene have been identified (16, 93, 97, 113, 130, 215, 218). CD14 polymorphisms have been found to affect not only serum levels of sCD14 but also IgE (16, 218). This raises the possibility that these genetic variants may affect not only innate immunity but adaptive immunity as well. Although the presence of CD14 variant alleles has been linked to disorders that may have an infectious etiology, there are no reports examining the relationship to periodontitis (97, 215). Levels of CD14 present in gingival crevicular fluid have been associated with severity of disease (99), although a genetic involvement was not assessed.

Polymorphisms in both toll-like receptor 4 and toll-like receptor 2 have been identified in the human genome (103, 136, 181, 192). Some workers have attempted to find a link between these variants and a predisposition toward a number of disorders associated with bacterial infection. Although one would not expect to see the obvious impairment of host defense to infections as seen in toll-like receptor-deficient mice, it may be that altered toll-like receptor proteins may have mildly decreased functionality or exhibit dysfunctionality to a subset of microorganisms relevant to a particular disease. Kang & Chae (103) found an association of the presence of a single base substitution in toll-like receptor 2 with lepromatous leprosy but not tuberculoid leprosy, indicat-

ing a role for toll-like receptor 2 in susceptibility to lepromatous leprosy. Another group (136) found an association between a polymorphism in toll-like receptor 2 and susceptibility to staphylococcal infections in sepsis patients. However, no association of a specific toll-like receptor 4 polymorphism with meningococcal disease was seen (181). Based on these early results, it seems that variants of toll-like receptor genes may or may not affect the health of the individual possessing the allele. In the future, studies with large sample sizes will be required to reveal or clarify the existence of a possible link or association between polymorphisms within the toll-like receptors and specific disease states.

Conclusions

A critical goal of the host is to develop a *dynamic* state of health in which a continued bacterial challenge is countered with an appropriate innate host response that leads to bacterial clearance. Clearance of bacteria can occur due to the specificity within the innate immune system that results from the consensus of the variety of receptors and their specific ligands. Some of these innate receptor–ligand complexes are involved in bacterial presentation; some are involved in host cell activation. Although the innate response is inherently rapid, multiple host proteins are required and are exquisitely involved with these interactions with microbial components, each host protein–ligand complex possessing different specificities. Elucidation of these interactions is further complicated by variation in the *in vivo* expression with time, environmental stresses, osmotic and fluid changes that are difficult to duplicate or recreate *in vitro*.

Bacterial clearance can also occur without further activation of host response(s) or can activate specific adaptive immune responses that are also designed to "re-establish" health. In addition, although not formally examined, the more severe the deficiency in neutrophil chemotaxis, migration, accumulation or phagocytosis (seen in congenital diseases), the less the *need* or *requirement* for a pathogenic bacteria to initiate or alter the severity of disease. In contrast, in a healthy periodontium and host, specific pathogenic bacteria (in an attempt to evade and survive within the host) seem to possess the ability to induce specific innate defects that render the host susceptible to disease. For example, neither *P. gingivalis* lipopolysaccharide, whole cells nor isolated cell walls were able to facilitate E-selectin expression in human endothelial cells, suggesting that *P. gingivalis* is a natural antagonist of E-selectin expression (39). This disrupts one of the first committed steps in the diapedesis and extravasation of leukocytes from the vasculature compartment into the surrounding tissue. In addition, *P. gingivalis* lipopolysaccharide was also found to inhibit the expression of E-selectin and neutrophil adhesion in response to other bacteria. Furthermore, *P. gingivalis* has been shown to disrupt the ability of gingival epithelial cells to secrete IL-8 in response to other bacteria (40). We hypothesize that these factors, coupled with genetic polymorphisms, could act separately or synergistically, creating a dysfunctional host inflammatory response that disables the protective mechanisms of the host, causing susceptibility to a variety of chronic periodontal conditions (Fig. 1C).

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Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease

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Over the past decade, numerous studies have attempted to delineate the Th1/Th2 cytokine profile in chronic periodontitis in humans. While some controversy still exists regarding these profiles, the fact that the stable periodontal lesion is identical to a delayed type hypersensitivity reaction while the progressive lesion involves large numbers of B cells and plasma cells, strongly suggests that the stable lesion is mediated by Th1 cells and the progressive lesion by Th2 cells. The control of Th1 and/or Th2 expression is therefore fundamental in understanding the immunoregulatory mechanisms in chronic periodontitis. In this context, high affinity T cell receptor (TCR) interactions seem to direct a Th1 response while low affinity TCR involvement tends towards a Th2 response. As yet however, TCR affinity in chronic periodontitis has not been investigated. Other mechanisms which may control Th1/Th2 profiles include the nature of the antigen(s), antigen presentation and the innate immune response. The role of these mechanisms in periodontal disease is reviewed and suggestions for future research are put forward.

Introduction

Chronic inflammatory periodontal disease results from the inflammatory response to bacteria in dental plaque and may either remain confined to the gingival tissues, or progress leading to attachment loss endangering the life of the dentition (Fig. 1). Disease progression is due to a combination of factors including the presence of periodontopathic bacteria, high levels of proinflammatory cytokines, matrix metalloproteinases and prostaglandin E_2 (PGE_2) and low levels of inflammation inhibitory cytokines including interleukin (IL)-10, transforming growth factor (TGF)-β and tissue inhibitors of metalloproteinase (141). In some individuals, neutrophils and cell mediated immunity may limit the extent of attachment loss. However, in susceptible people as determined by genetic and environmental factors, the presence of defined periodontopathic bacteria such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* or *Tannerella forsythia* may limit clearance by neutrophils and disease progression may occur. The adaptive immune response is under the control of T cells which regulate B cell/plasma cell differentiation and antibody production. Clearance of bacteria by neutrophils may depend upon the presence of interferon (IFN)-γ and may be further enhanced by protective antibodies which in turn are controlled by the types of cytokines produced by T cells (reviewed in 141).

T cells and cytokines in periodontal disease

The development and regulation of an immune response depends to a large extent on the local production of a number of cytokines which can determine whether the response will be a protective or non-protective one. The immune response to infection is regulated by the balance between T helper (Th)1 and Th2 cytokines. The net effect of the Th1 cytokines IL-2 and IFN-γ is to enhance cell mediated responses, while that of the Th2 cytokine IL-4 is to suppress cell mediated responses and hence enhance the resistance associated with humoral immunity (130).

It is evident that both T and B cells are present in periodontal disease tissues (212), the majority of T cells being activated memory/primed cells

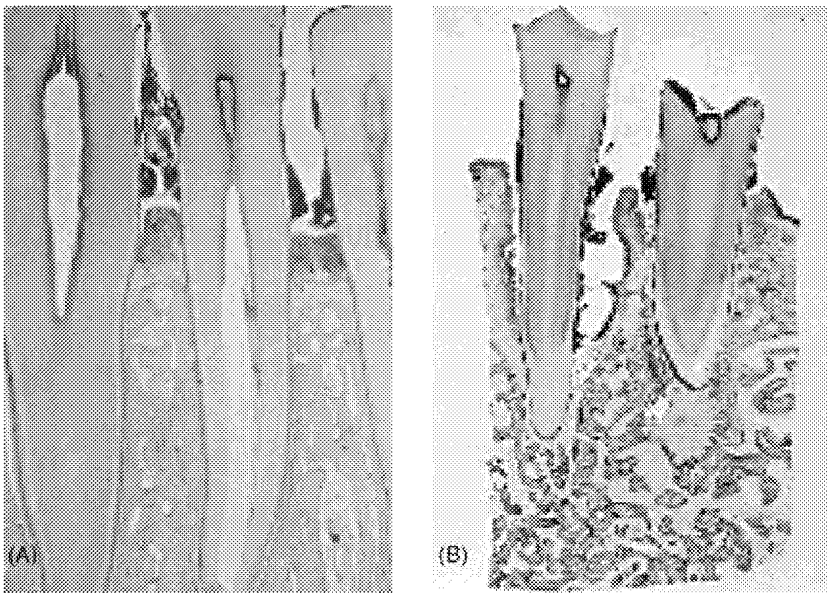

Fig. 1. (A) Inflammation confined to the gingival tissues with minimal tissue destruction. (B) Extreme periodontal destruction with loss of attachment and alveolar bone.

(61, 138, 206). Both T and B cells extracted from gingival tissues have been reported to be at a more advanced stage of the cell cycle than peripheral blood T and B cells, indicative of activation within the tissues (57) or the selective extravasation of activated cells. The infiltrate in the periodontal lesion consists of lymphocytes and macrophages and it has been hypothesized that T lymphocytes predominate in the stable lesion, while the proportion of B cells and plasma cells is increased in the progressive lesion (107, 116, 155, 172–174) (Fig. 2). This has prompted the suggestion that T cells with a Th1 cytokine profile may be the major mediator in the early/stable lesion. The production of IFN-γ would enhance the phagocytic activity of both neutrophils and macrophages and hence containment of the infection. However, the lesion persists due to the continual formation of the plaque biofilm (59). The dominance of B cells/plasma cells in the advanced/progressive lesion would suggest a role for Th2 cells. If the innate response is poor, low levels of IL-12 would be produced and a poor Th1 response may occur, which may not then contain the infection. Mast cell stimulation and the subsequent production of IL-4 would encourage a Th2 response, B-cell activation and antibody production. If these antibodies

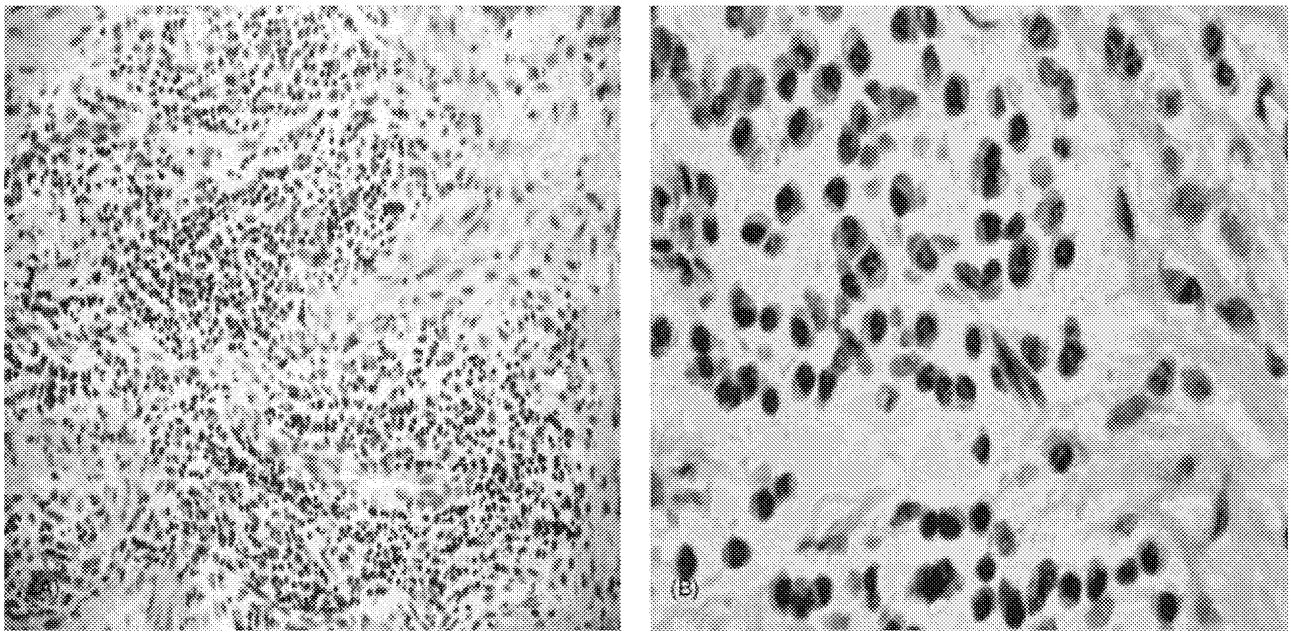

Fig. 2. (A) Perivascular lymphocytic/macrophage infiltrate in a gingivitis lesion. (B) Plasma cells in an advanced periodontitis lesion.

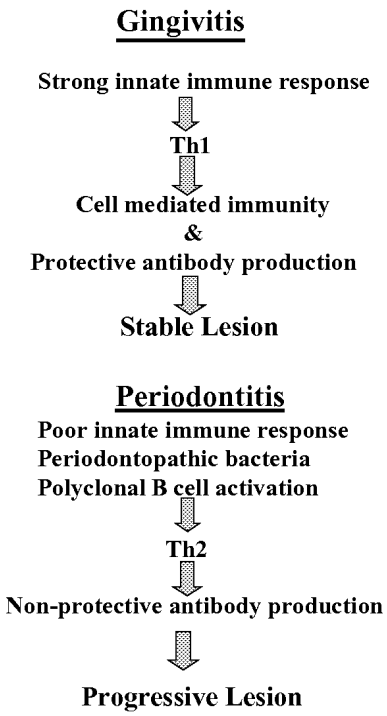

Fig. 3. Model for Th1/Th2 regulation in the stable and progressive lesion.

are protective and clear the infection, the disease will not progress but if on the other hand they are non-protective, the lesion will persist and continued B-cell activation would result in large amounts of IL-1 and hence tissue destruction (59, 63, 175) (Fig. 3).

A number of studies have attempted to delineate the Th1/Th2 profile in periodontal disease. Decreased Th1 cytokines in the gingival crevicular fluid (146) and gingival mononuclear cells (49) of periodontitis sites, peripheral blood mononuclear cells from periodontitis patients stimulated with mitogens (178), *P. gingivalis* and *Fusobacterium nucleatum* (59) have been demonstrated. Increased Th2 responses in periodontitis have been reported in studies on peripheral blood (4, 14, 207), gingival tissues (105, 192, 208), extracted gingival mononuclear cells (122) and gingival crevicular fluid (156). These studies support the hypothesis that Th1 cells are associated with the stable lesion and a Th2 response with disease progression. However, other studies have reported results consistent with the predominance of Th1-type cells or reduced Th2 responses in diseased tissues (43, 163, 187).

Most recent studies have suggested the involvement of both Th1 and Th2 cells in periodontal disease. mRNA for both Th1 and Th2 cytokines has been demonstrated in studies of cells extracted from periodontal lesions (50, 150). A role for IL-10 was suggested by another study which demonstrated two distinct profiles of cytokine expression in $CD4^+$ gingival lymphocytes isolated from inflamed periodontal tissues: in both, IFN-γ, IL-6 and IL-13 mRNA were present, but in only one was IL-10 mRNA present. In most cases, IL-2, IL-4 and IL-5 mRNA were not detected (205). IL-10 has been demonstrated to inhibit lipopolysaccharide-induced B-cell proliferation in the mouse (123) such that decreased IL-10 in periodontitis may possibly allow continued polyclonal B-cell activation. Reports of comparisons between gingival tissues and peripheral blood of periodontitis (209) and *P. gingivalis*-stimulated peripheral blood mononuclear cells from periodontitis and gingivitis patients (133) also demonstrated a role for both Th subsets. In the last study, there was no correlation with disease status or the presence of *P. gingivalis* in the plaque. Reports on *P. gingivalis*-specific T-cell lines and clones have also demonstrated conflicting results (62, 64, 91, 198); in addition, Wassenaar et al. (199) showed functional differences in $CD8^+$ T-cell clones. Those that produced high levels of IFN-γ but no IL-4 or IL-5 (Th1) mediated cytolytic activity. Other CD8 clones produced high levels of IL-4 together with IL-5 and displayed no cytotoxicity but could suppress the proliferative response of cytotoxic CD8 T-cell clones. It was concluded that $CD8^+$ T cells may participate in the local response by suppressing IFN-γ producing cells and favoring humoral immune responses.

The results of all these studies are difficult to interpret due to differences in the material examined, the methodologies used, the use of different sources of cells including cells extracted from gingival tissues, peripheral blood mononuclear cells, T-cell lines and clones and the *in vitro* use of various components of different bacterial strains (212). However, it is likely that different T-cell subsets predominate at different phases of disease and the inability to determine disease activity clinically is a major limitation in all these studies.

Chemokines in periodontal disease

The regulation of leukocyte migration into and through the tissues is determined by the expression of adhesion molecules on endothelial cells and other cells such as keratinocytes, which are induced by pro-inflammatory cytokines as well as to a group of cytokines with chemotactic properties, the chemokines. Chemokines are responsible for the recruitment and subsequent activation of particular leukocytes into inflamed tissues (9) and therefore

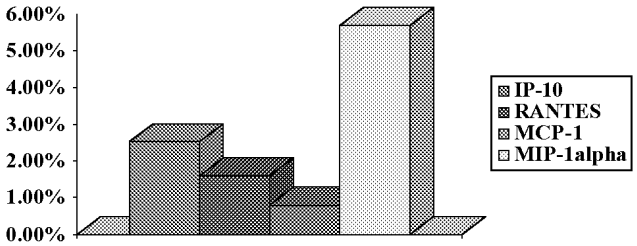

Fig. 4. Chemokine expression in periodontitis tissues compiled from Gemmell et al. (67).

play a central role in the final outcome of the immune response by determining which subsets of leukocytes form the inflammatory infiltrate. Th1 and Th2 cells differ in their migratory properties and chemotactic responsiveness so that chemokines may regulate local immune reactions by influencing the local balance of T-cell subsets (179).

Recently, MCP-1 (monocyte chemoattractant protein-1) production by monocytes has been shown to be regulated by IL-10 (196). Decreased numbers of IL-10^+ T cells extracted from adult periodontitis lesions compared with cells extracted from gingivitis tissues have been demonstrated (60). Furthermore, the percentages of MCP-1^+ T cells, B cells and monocytes in *P. gingivalis*-specific T-cell lines have been reported to be reduced compared with other chemokine positive cells (65), and in an immunohistological study, very few MCP-1^+ cells were demonstrated in inflamed gingival tissues (67) (Fig. 4). In contrast, Yu et al. (211) reported the expression of MCP-1 on endothelial cells as well as monocytes/macrophages in inflamed gingival tissues correlated with the degree of inflammation. However, an animal model study showed MCP-1 was reduced or absent in mice 4 h after receiving intramuscular injections of *P. gingivalis* lipopolysaccharide (154). Overall, these results suggest that reduced IL-10 in periodontitis could result in reduced MCP-1 and cell-mediated responses.

MCP-1 has been suggested to contribute more to type 2 than to type 1 cytokine-mediated inflammation (29, 76, 114). However, another chemokine, MIP-1α (macrophage inflammatory protein-1α) has been reported both to shift the immune response to a Th2-type response (97) and to recruit Th1 cells (179). Immunohistochemistry has shown higher numbers of MIP-1α positive leukocytes in periodontal disease tissues (67) (Fig. 5). Microchemotaxis experiments have shown MIP-1α to be a potent chemoattractant for B cells and cytotoxic T cells, although at higher concentrations the migration of CD4 cells was enhanced (168). Both MIP-1α (42, 106) and MCP-1 (88) have been shown to be involved in the recruitment of neutrophils. Gingival keratinocyte expression of MIP-1α has been demonstrated to be increased in comparison with IP-10 (IFN-γ inducible protein 10), RANTES (Regulated on Activation Normal T cell Expressed and Secreted) or MCP-1 and while the expression of the latter 3 chemokines decreased with increasing inflammation, that of MIP-1α did not (67). This suggests a role for MIP-1α in the recruitment of leukocytes through the epithelium at early as well as later stages of

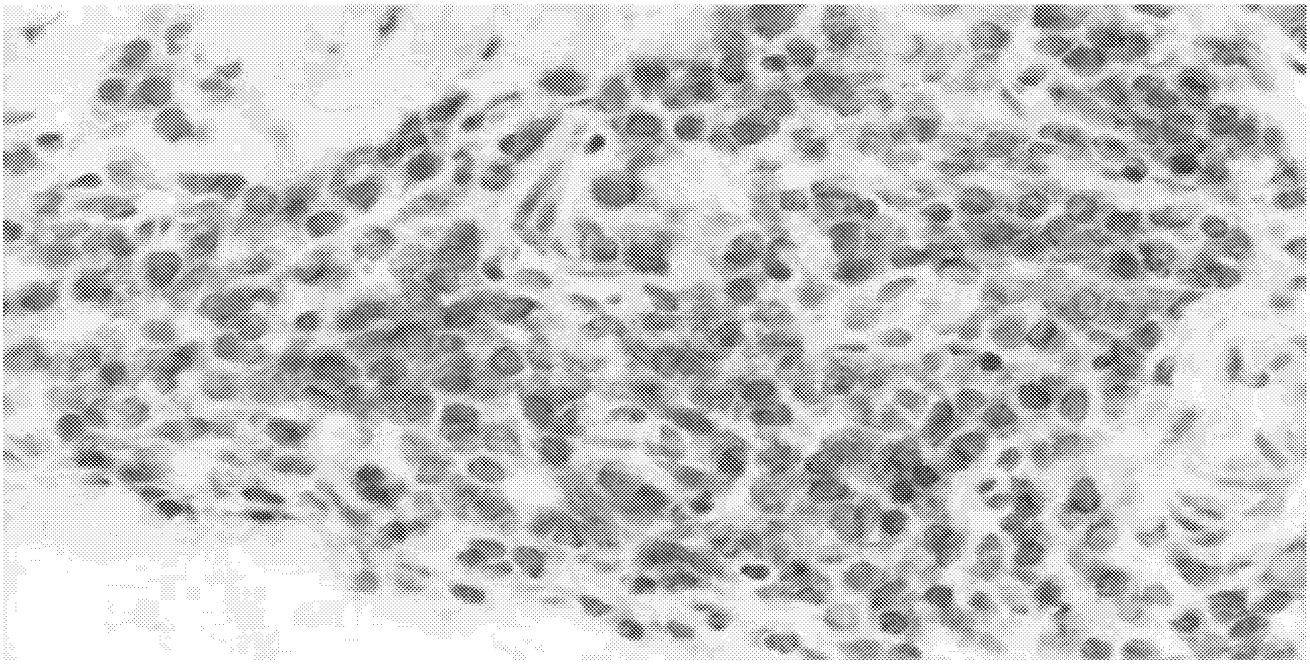

Fig. 5. MIP-1α expression in chronic periodontitis tissue.

inflammation (67). In contrast, Tonetti et al. (193) demonstrated mRNA for MCP-1 in tissue biopsies taken from clinically healthy sites, sites undergoing a 21-day experimental plaque accumulation and from untreated and treated periodontitis sites. MCP-1 mRNA was detected in the oral epithelium particularly the basal layer, as well as in the inflammatory infiltrate in periodontal disease tissues. It was noted that while MCP-1 expression related to mononuclear phagocytic infiltration in the connective tissues and oral epithelium, macrophages were consistently present in the junctional epithelium in the absence of MCP-1 message indicating the presence of other mediators responsible for the infiltration of these cells.

The role played by RANTES in the migration of T-cell subsets and Th1/Th2 cells is controversial. RANTES was recently detected in the gingival crevicular fluid of patients with periodontitis but not subjects with clinically healthy gingiva and the concentration was significantly higher in samples from active sites than in those from inactive sites (54). Furthermore, RANTES levels decreased after periodontal therapy suggesting a relationship between this chemokine and periodontal disease status (53). IP-10 on the other hand is specific for activated T cells. It was shown to target Th1 cells selectively resulting in the upregulation of IFN-γ rather than IL-4 peripheral blood producing T cells (45, 55). However, an immunohistological study reported no differences in IP-10 or RANTES in the gingival tissues suggesting no predominant T-cell subset recruitment by these chemokines in gingivitis or periodontitis (67).

Taubman and Kawai (189) have recently demonstrated that Th1-type T cells which preferentially express CCR5 and CXCR3 are found predominantly in diseased gingival tissues whereas little CCR5 expressed by Th2 cells could be found. It was also shown that the chemokine ligands RANTES and MIP-1α (CCR5) and IP-10 (CXCR3) were elevated in inflamed periodontal tissues. These authors cite these results as supporting evidence for Th1 involvement in periodontal bone resorption. However, other factors which modulate T-cell cytokine profiles must be monitored to determine the nature of Th1/Th2 responses in periodontal disease.

B cells and antibody regulation in periodontal disease

B cells and plasma cells produce and secrete immunoglobulins which protect the host by various methods including prevention of bacterial adherence, inactivation of bacterial toxins and by acting as opsonins for phagocytosis by neutrophils. Antibodies can downregulate or up-regulate the immune response. If the result of B cell differentiation is protective antibody production, elimination of the causative organism would ensue and periodontal disease progression would stop. Production of non-protective antibodies in susceptible subjects, could on the other hand result in continual connective tissue breakdown. Periods of destruction would precede periods of stability and the disease may have a cyclical pattern with not all B cell lesions being destructive (59, 175).

The inability of specific antibodies to eliminate the causative organisms of periodontal disease could be due to a number of factors including poor antigenicity of virulence determinants and elicitation of antibodies with poor anti-bacterial properties (180). The production of anti-*P. gingivalis* antibodies with different avidities in various forms of periodontal disease have been suggested to reflect the quality of the humoral response which may affect progression of the disease (131). Non-protective low avidity anti-*P. gingivalis* antibodies may be incapable of effectively mediating a variety of immune responses (110, 201). A recent study in children by Morinushi et al. (132) showed that serum anti-*P. gingivalis* but not anti-*A. actinomycetemcomitans* antibodies were inversely correlated with gingival inflammation, suggesting an inhibition of *P. gingivalis* antibodies. Since these responses are regulated by immunoregulatory genes, it may be that antibody responses are protective in one individual but not in another (136).

During the chronic phase of the disease, the antibody response has been suggested to be generally protective, facilitating bacterial clearance and arresting disease progression (136). Anti-*P. gingivalis* protease antibodies which occur late in periodontitis infections have been demonstrated to block the anti-opsonizing activity against C3 and IgG (35). An increased capacity of serum to opsonize *P. gingivalis* has been shown to be a distinctive feature in patients with a past history of destructive periodontal disease (204). Opsonic IgG antibodies to *A. actinomycetemcomitans* which may facilitate neutrophil-mediated phagocytosis and be protective against this periodontopathic organism have also been demonstrated (10, 195). Repeated infection with *A. actinomycetemcomitans* has also been shown to elicit an anti-leukotoxin antibody that protects neutrophils from the leukocidal activity of the leukotoxin (195).

Early studies indicated that polyclonal B-cell activation was significant in the pathogenesis of periodontal disease. Recently, Champaiboon et al. (25) showed that sonicated extracts of *P. gingivalis* stimulated peripheral blood B cells to proliferate, but not T cells. The results of all these studies suggest that if specific antibodies with high avidity and of protective IgG subclasses to immunodominant antigens are formed, the infection may be cleared and the disease will not progress. If, however, polyclonal B-cell activation is induced by periodontopathic organisms and non-specific and/or low avidity specific antibodies are produced, the infection may not be cleared. Continued B-cell activation may lead to the production of high levels of IL-1, resulting in tissue destruction. This is consistent with the observations that B cells are a potent source of IL-1 (60), that macrophages are not a dominant feature of the advanced lesion (26) and that suppressed cell-mediated immunity is associated with advanced periodontitis (18, 86, 175).

The B-cell response requires T-cell help in the form of cell–cell contact as well as cytokines, which are responsible for the expansion and differentiation of B cells into plasma cells and in class switching (137). T cells are necessary for both specific antibody production and polyclonal B-cell activation (160) and as they are the dominant cell type in the cell-mediated (macrophage/lymphocyte) response, T-cell determination of the resulting antibody response must play a fundamental role in the pathogenesis of periodontal disease.

T-cell receptor affinity

Differentiation of Th1 and Th2 T-cell subsets is determined during priming and is influenced by a number of factors, including the cytokine environment, the antigen itself, antigen dose, the route of administration, the nature of the antigen presenting cell and costimulatory molecules. Recent studies have shown that the affinity of the major histocompatibility complex/peptide/T-cell receptor interaction determines the differentiation of $CD4^+$ cells into either Th1 or Th2 cells (20). In the presence of IL-12, a short T-cell receptor stimulation has been shown to induce Th1 polarization, IL-12 exerting its effect during and after T-cell receptor signaling. Th2 polarization, on the other hand, was found to require prolonged T-cell receptor signaling and IL-4 was effective only when present during T-cell receptor triggering (84). These authors concluded that the duration of T-cell receptor stimulation was crucial in influencing Th1/Th2 polarization. Furthermore, Busch & Palmer (23) showed that *in vivo* expansion of T cells after bacterial infection is accompanied by an increase in the T-cell affinity for antigen. T cells which have undergone a number of rounds of *in vivo* expansion have been demonstrated to express a narrower range of T-cell receptors and to bind major histocompatibility complex/peptide complexes with greater affinity. The strength of the T-cell receptor signal has also been found to determine the involvement of CD28 costimulation in CD4 T-cell differentiation. In this study, IL-4-producing Th2 cells were generated after priming with a weak T-cell receptor signal but not with a strong signal, and this was dependent on CD28/B7 interactions (188). It was concluded that a more sustained engagement of the T-cell receptor by peptide/major histocompatibility complex polarizes T-cell receptor transgenic splenocytes to a Th2 profile. Evidence of a correlation between T-cell receptor affinity and cytokine profiles has been put forward in a study using a *Leishmania major* mouse model. T cells from susceptible and resistant mice expressed low- and high-affinity T-cell receptor, respectively, the T cells from resistant mice producing more IFN-γ and less IL-4 than those from susceptible mice. This suggests an association between high affinity T-cell receptor and Th1 cells (120).

The impact of T-cell receptor selection and structure has been studied in Th1 and Th2 lines and clones with fixed peptide specificity and class II restriction (20). The Th2 clones tended to use longer T-cell receptor complementarity-determining region (CDR)3α loops than their Th1 counterparts. Molecular modeling of Th1- and Th2-derived T-cell receptors showed that Th2 CD3α comprised larger side chain residues than Th1 T-cell receptors. It was proposed that under Th2 polarizing conditions, there was a trend for $CD4^+$ T cells to have elongated T-cell receptor CD3α loops, which are predicted to alter T-cell receptor binding and reduce contact at other interfaces, possibly impeding T-cell receptor triggering. Boyton et al. (20) concluded that either the elongated receptor was lost during selective expansion of Th1 cells or that selection of the Th2 line was compatible with expansion of cells bearing either type of receptor, with the elongated form as the preferred receptor.

Boyton & Altmann (19) have proposed that differential selection from the available pool of specific T-cell receptors occurs during Th1 or Th2 development. They also suggest that programming to select

for cells to become either Th1 or Th2 T cells may come from local factors such as the cytokine or chemokine milieu. They also state that while many factors determine the polarization of T cells, any one factor can override the changes initiated by any other factor. Therefore, while antigen dose or the type of antigen presenting cell is important, exogenous cytokines can polarize the resultant response (19).

Regulatory T cells

While Th1 and Th2 subpopulations of T cells determine the response to infection based on the cytokine pattern induced, distinct so-called regulatory T cells with immunosuppressive function and different cytokine profiles have been described that may prevent infection-induced immunopathology or prevent pathogen elimination by suppressing protective Th1 responses (128). Three distinct regulatory T cell (Tr) subsets have been described. Tr1 CD4 cells secrete high levels of IL-10 and low to moderate levels of transforming growth factor (TGF)-β and have been shown to prevent the development of Th1-mediated autoimmune diseases and suppress immune responses to pathogens, tumours and alloantigens (75). The suppressive effects of Tr1 clones are reversed by IL-10 neutralization, suggesting that, regardless of T-cell antigen-specificity, Tr1 suppression is a bystander effect mediated by IL-10 (127). Th1 and Th2 cells reciprocally regulate the other subpopulation by the secretion of IFN-γ and IL-4 and also possibly by IL-10 via a negative feedback loop. Tr1 cells which secrete high levels of IL-10 can suppress Th1 responses to an infectious pathogen (127). Certain Tr1 cells have also been reported to suppress Th2 responses (34).

The second subset of regulatory T cells are Th3 CD4 cells, which primarily secrete TGF-β (51). As this cytokine is secreted by many cell types, Th3 cells may have a major role in immune regulation (128). TGF-β is an important anti-inflammatory agent and IL-1 inhibitor (145). It has been reported that, on extraction, no TGF-β^+ gingival mononuclear cells, as determined by FACS analysis, could be detected (58). Stimulation with either *P. gingivalis* or *F. nucleatum* resulted in a transient rise in the percent of positive cells. Unlike the gingival cells, small numbers of TGF-β^+ peripheral blood cells were present on extraction. After stimulation, the percentage of positive cells rose continuously for the 6 days of culture. Th3 CD4 cells may therefore not be a significant subset of regulatory cells in periodontal disease.

$CD4^+$ $CD25^+$ T cells make up approximately 5–10% of the peripheral blood T-cell pool and immunosuppression occurs by inhibition of IL-2 production, via a mechanism dependent on cell–cell contact as well as the expression of CTLA-4, which is a CD28 homolog and negative regulator of T-cell activity (153, 191). $CD4^+$ $CD25^+$ Tr cells may use multiple mechanisms to mediate suppression (128). In regard to periodontal disease, an immunohistological study of experimental gingivitis in humans demonstrated very few $CD25^+$ cells in the inflammatory lesions and it was not determined whether the small numbers of positive cells were T cells (174). This indicates a limited role for $CD4^+$ $CD25^+$ T cells in periodontal disease, although the expression of CD25 on T cells has not yet been determined in progressive periodontal lesions. However, there is controversy concerning the use of CD25 as a marker for Tr cells and pathogen-specific Tr cells have been suggested to become mature only after repeated antigen stimulation and to exert their regulatory role at the end stages of infection (128). Surface markers expressed only on Tr cells must be identified to understand fully the function and relationship of distinct Tr cell subsets (128).

The development and persistence of chronic infections have been postulated to be due to an imbalance of either a Th1 or a Th2 profile, although suppression of a protective immune response by regulatory T cells may be a major factor (128). Pathogen-stimulated IL-10 or TGF-β by innate cells including macrophages and dendritic cells may suppress the immune response early in infection, this suppression being maintained by the induction of Tr1 or Th3 cells (128). Pathogens which cause chronic infections may export Tr cells to counteract protective Th1 responses, which in turn will prolong survival (128).

Although Tr cells, like Th1 or Th2 cells, arise from naïve precursors, McGuirk & Mills (128) question whether subpopulations of antigen-presenting cells may then promote the differentiation of regulatory rather than effector T cells. Activation of dendritic cells which secrete IL-10 but not IL-12 have been reported to direct T cells to a Tr1 subtype (1, 127). Functional subpopulations of dendritic cells may be activated via distinct signals from pathogen-derived molecules rather than different lineages *in vivo* and other innate cells (128).

Understanding the role of Tr cells will impact on our understanding and treatment of disease. The ability to induce certain T-cell subsets with specific cytokine profiles will enable the design of therapies

for prevention and treatment of chronic infections (128).

Coinfection studies

Dental plaque is a complex biofilm. Adhesion of colonizing organisms to the enamel salivary pellicle occurs initially, followed by secondary colonization via inter-bacterial adhesion (reviewed in 159). Subgingival plaque, which resides in a more protected location than supragingival plaque, is not subject to the same degree of intraoral abrasion or salivary host defense mechanisms and, as with biofilms in general, is very resistant to removal (38). Retention and growth of bacteria is facilitated by a number of adhesins including fimbriae, hemagglutinins and proteinases (104). *P. gingivalis* binding occurs via fimbrillin, the structural subunit of the major fimbriae. This asaccharolytic organism requires hemin and peptides for growth, producing at least three hemagglutinins and five proteinases for these requirements. The hemagglutinins participate in adherence interactions and the proteinases contribute to adverse immune responses and to tissue destruction. *P. gingivalis* also modulates host cell signal transduction pathways, resulting in uptake by gingival epithelial cells where the bacteria can influence components of the innate immune system (104).

Holt et al. (80) concluded in a study on the nonhuman primate *Macaca fascicularis* that while a selected group of organisms may be important in the disease process, other members of the microbiota may play a significant role in the events leading to disease progression. The commensal nature of many of the bacteria in the complex biofilm must be taken into account in studies of antigenic epitopes of periodontopathogens and the immune responses elicited in the host (147). Therefore, while periodontopathic bacteria such as *P. gingivalis* and *T. forsythia* are essential for periodontal disease progression to occur, interactions between the many species of oral bacteria must be considered to be important factors in the development of periodontal disease. Many studies have reported the bacterial composition associated with health, gingivitis and periodontitis (reviewed in 38). However, as stated above, studies of antigens associated with periodontal disease have provided conflicting results due to the variable nature of the composition in individuals.

Animal models have been used to study the pathogenicity of mono-infections of periodontopathogens such as *P. gingivalis* (27, 72, 93, 96, 100). These studies examined the effect of immunization with invasive and non-invasive strains of *P. gingivalis* in the clearance of organisms, healing of lesions and production of specific antibodies as well as protective or non-protective isotypes. In recent years, reports have also been published on the effects of mixed microbial infections including *P. gingivalis* and *A. actinomycetemcomitans* (28), *P. gingivalis* and *F. nucleatum* (43) and *P. gingivalis* and *T. forsythia* (210). The results of these studies generally demonstrated increased levels of pathogenicity with a synergistic effect observed in the humoral and cellular host responses. Another study demonstrated an inhibition of serum anti-*P. gingivalis* antibodies when *F. nucleatum* immunization preceded that of *P. gingivalis,* with significantly reduced levels of both anti-*P. gingivalis* and anti-*F. nucleatum* antibodies when *P. gingivalis* immunization preceded that of *F. nucleatum,* indicating inhibitory effects on antibody production by both bacteria (69). Both *P. gingivalis* and *F. nucleatum* induced Th1 and Th2 responses and while *F. nucleatum* did not have an effect on the T-cell cytokine profiles induced by *P. gingivalis*, the percentage of IL-4^+ CD4 cells in the spleens of mice immunized with both organisms was increased compared with the control group, indicative of a Th2 response (69). In another study, Choi et al. (31) established *P. gingivalis*-specific T-cell clones from the spleens of BALB/c mice immunized with *P. gingivalis* and *F. nucleatum* and found that lines from mice injected with *P. gingivalis* had a polarized Th1 profile, whereas those from mice immunized with both *bacteria* had a polarized Th2 cytokine pattern. It was concluded that *F. nucleatum* provided an immunomodulatory role in that T cells initially primed by *F. nucleatum* antigens may result in the development of Th2 cells. Primary infection of mice with *P. gingivalis* and *F. nucleatum* has also been shown to result in significantly greater lesion size compared with infection with *P. gingivalis* alone (46). However, when the ratio of *F. nucleatum* to *P. gingivalis* was 1 : 1 or greater, the spread and progress of lesions was decreased, suggesting an inhibitory effect on the virulence of *P. gingivalis*, although infection with *F. nucleatum* prior to or 1 h after *P. gingivalis* infection enhanced the ability of *P. gingivalis* to form large phlegmonous lesions. Following on from this study, Ebersole et al. (43) showed that active immunization of mice with *P. gingivalis* protected against challenge with *P. gingivalis* as well as *P. gingivalis* together with *F. nucleatum* and this protection, in terms of lesion size, correlated with the levels of specific serum IgG antibody.

It is now being recognized that bacteria within biofilms have properties which may not be seen when individual species are grown independently (73). Future animal studies must use biofilms formed using initial colonizing plaque bacteria together with periodontopathic organisms as immunogens in order to mimick more closely the immune response involved in periodontal disease.

Susceptibility to periodontal disease

Innate susceptibility to periodontal disease is influenced by host genotype (129). Genetic polymorphisms in Fc receptors on phagocytic cells may be significant in determining susceptibility to bacterial infections (164). Individuals with low affinity Fc receptors for IgG2 (Fcγ RIIa) have reduced IgG2-mediated phagocytosis of encapsulated bacteria and are susceptible to a variety of bacteria including meningococcal infections (21, 165). Serum IgG2 antibodies are the predominant response to *P. gingivalis* antigens (110, 148, 201, 204), although a recent study found no association in the frequency of Fcγ RIIa or Fcγ RIIIa receptor haplotypes with refractory or successfully treated periodontal disease or periodontally healthy individuals (33).

Cytokine polymorphisms have been reported to influence the immune response in periodontal disease. Galbraith et al. (52) demonstrated a correlation between the TNF-α 308 genotype and levels of TNF-α production by oral neutrophils in periodontitis patients with a significant association between the T1,2 genotype and patients with advanced disease. However, another study found no correlation between genetic polymorphisms and periodontal disease (177). Similarly, no evidence of a role for IL-10.G alleles in genetic susceptibility to early-onset periodontal diseases could be demonstrated (78). On the other hand, IL-1 polymorphisms have been claimed to be a risk factor for severe periodontal disease with genotype positive individuals (101). Furthermore, an IL-1 genotype (IL-1 β +3954) in combination with smoking as well as a combined IL-1 β and IL-1 receptor antagonist (IL-1RA) genotype have been shown to be risk factors, supporting a role for both genetic and environmental factors in susceptibility to early-onset periodontitis (142). Another study demonstrated that the composite IL-1 genotype including allele 2 at each of two loci (IL-1A +4845 and IL-1B +3954) was associated with severity of periodontitis and that, again, both genotyping and smoking history could provide risk factors for periodontal disease (126). A more recent report suggested an active role of IL-2 in the pathogenesis of periodontal disease by the finding of an association in the −330 (T → G) polymorphism in the IL-2 gene with periodontal disease severity (167).

Neuroendocrine regulation may be significant in periodontal disease susceptibility. Lymphoid organs are richly innervated, suggesting direct contact between neurons and leukocytes. Leukocytes express receptors to neurotransmitters, especially noradrenalin, as well as receptors for several hormones produced by endocrine glands, including corticosteroids, which have immunosuppressive properties. Other receptors are specific for endorphins, growth hormone, thyroid hormone, substance P and ACTH, some of which are produced in small quantities by the cells of the immune system themselves. On the other hand, many cytokines produced by leukocytes can act on neurons and endocrine glands including IL-1, IL-6 and TNF-α (98). A recent experimental periodontitis rat model of hypothalamic pituitary adrenal axis hyperreactivity suggested that central nervous system regulation of immune responses to dental plaque bacteria may modulate susceptibility and disease progression (22).

While periodontopathic bacteria and the inflammation they provoke are essential for disease progression, environmental risk factors such as tobacco smoking, psychosocial stress and systemic diseases such as diabetes modify the host response and may be major determinants of the enormous variation in susceptibility (141). These factors may modify the pathways by which bacteria cause inflammation and hence modify disease progression, severity and outcome (141). Periodontal disease has been cited as a major complication of diabetes, with patients displaying an increased incidence and severity of disease (83) and poor periodontal health of pregnant women has recently been cited as a potential risk factor for low birth weights (39). Some risk factors are a concern for periodontal disease and certain systemic diseases such as cardiovascular disease (32). Individuals with severe periodontitis have been reported to have a significantly increased risk of developing cardiovascular disease including atherosclerosis, myocardial infarction and stroke (40). Various mechanisms have been proposed to explain the association between periodontal and cardiovascular disease. Although one pattern of IL-1 genetic polymorphisms as described above is associated with periodontitis, another pattern characterized by the IL-1B (−511) and IL-1RN (+2018) markers is associated with atherosclerotic plaque formation but not

periodontitis. Models are currently being formulated to show how IL-1 genetic factors may be involved in cardiovascular disease (103).

Recently, Wick et al. (202) investigated the induction of increased T-cell infiltration in early atherosclerotic lesions in a rabbit model. All immunized animals developed arteriosclerotic lesions at the predilection sites regardless of the immunogen used and the inducing factor was determined to be mycobacterial heat shock protein 65. Heat shock proteins are induced by cells on exposure to various forms of stress including temperature, oxidative injury, irradiation, infection and heavy metals and participate in physiological processes such as the assembly, transport and protection of proteins from denaturation (149). There is a remarkable conservation in the structure of heat shock genes and heat shock protein across species. During infection, bacterial heat shock proteins constitute major antigen determinants (94). The immune system may not be able to differentiate between self-heat shock protein and bacterial heat shock protein; cross-reactive epitopes of T cells with specificity for self-heat shock protein can therefore be activated during infection and antibodies generated by the host directed at pathogenic heat shock protein could result in an autoimmune response to similar sequences of the host (149). Significantly increased levels of anti-heat shock protein 65 antibodies have been reported in clinically healthy humans with sonographically demonstrable atherosclerotic lesions in their carotid arteries, compared with individuals who did not have these lesions (202). These antibodies were cross-reactive with heat shock protein 60 of other bacteria including GroEL of *Escherichia coli* and there was a correlation between high antibody titers and high mortality. These antibodies also recognized human heat shock protein 60 and were able to lyze stressed but not unstressed endothelial cells. Endothelial cells in stressed areas of arteries, but not veins, express heat shock protein 60 and Wick et al. (202) proposed that oxidized low density lipoproteins may act as stressors to induce the expression of heat shock protein 60 and adhesion molecules by endothelial cells, resulting in interaction with heat shock protein 60/65-specific T cells. The presence of risk factors such as high blood cholesterol would then result in progression to severe and irreversible atherosclerotic alterations.

Multiple infectious agents have been detected in atherosclerotic plaques including *P. gingivalis* (30). GroEL-like proteins in several pathogenic bacteria have been reported to be major antigens (200) and an *E. coli* GroEL homolog identified in *P. gingivalis* (81, 113, 118) was shown to be immunogenic, being recognized by serum samples from periodontal disease patients (118). Western blot analysis demonstrated a higher positive response to *P. gingivalis* GroEL in periodontal disease patients than in healthy subjects (184) and anti-*P. gingivalis* GroEL antibodies were detected in all samples of inflamed gingival tissues. The concentration of IgG antibodies in the gingival tissue extracts was higher than in the corresponding serum samples, suggesting the antibodies were produced locally. Gingival homogenate samples from patients with adult periodontitis also reacted with anti-human heat shock protein 60 (3) with heat shock protein 60 expression demonstrated to be higher in gingival epithelial cells in inflamed tissues than in healthy samples (115). While these results suggest that both self and bacterial heat shock proteins may play a role in the development of periodontal disease, a more recent report has shown that the proliferative responses of peripheral blood mononuclear cells from periodontitis patients to mycobacterial and human heat shock protein 60 and 70 were lower than responses by cells from gingivitis subjects, suggesting that poor reactivity to heat shock protein may be a susceptibility factor for destructive periodontal disease (143). Similarly, Lopatin et al. (111) found that patients with higher anti-heat shock protein 90, DnaK and GroEL serum antibody concentrations tended to have significantly healthier periodontal tissues, particularly when the relationship between mean probing depths and antibody concentrations was analyzed. Additionally, with respect to anti-heat shock protein 90 antibodies, an inverse relationship with probing depth and positive relationship with colonization by *P. gingivalis* was found. It was concluded that an inability to mount an immune response to specific heat shock protein might identify patients at risk of developing periodontal disease.

Animal models have been used to determine the importance of genetic factors in periodontal disease. Baker et al. (13) concluded that susceptibility to bone loss occurring after oral infection with *P. gingivalis* is genetically determined. In another study, an inverse relationship in the splenic T- and serum B-cell responses to *P. gingivalis* was shown in strains of mice with different H-2 haplotypes (66), such that strong T-cell cytokine responses were accompanied by weak specific anti-*P. gingivalis* antibody levels and vice versa. Additionally, the levels of anti-*P. gingivalis* antibodies present in the serum samples appeared to correlate with healing of the lesions after subcutaneous challenge, higher levels being present

in mice which exhibited the fastest kinetics with regard to lesion recovery, indicating the significance of protective specific antibodies.

A recent study which examined the cytokine profiles of *P. gingivalis*-specific T-cell lines established from four mouse strains showed that the T-cell response to *P. gingivalis,* and in particular the CD4 response, may depend on major histocompatibility complex genes, further implicating genetic factors in the T-cell response in periodontal disease. The specific antibody response to *P. gingivalis* requires the presence of T cells (16) and T cells have been shown to influence periodontal bone destruction (11, 12). Recently, Katz & Michalek (92) adoptively transferred splenic or Peyer's patch T cells from normal Fischer rats orally infected with *P. gingivalis* into nude Fischer rats and found that after oral challenge with live organisms, serum and salivary responses were noted only in treated nude rats compared with control rats which did not receive T cells, confirming a T-cell-dependent response to *P. gingivalis*. Furthermore, higher serum IgG, especially IgG2, was correlated with less horizontal bone loss in these adoptively transferred rats. IgG2 responses indicate the involvement of Th1 cells, suggesting that this T-cell subset may be associated with a more favorable outcome in relation to bone loss after oral challenge to *P. gingivalis*.

Antigen-presenting cells

Different antigen-presenting cells have been suggested to direct T cells to a Th1 or Th2 pathway due possibly to presentation of different antigenic epitopes involving different second signals resulting in the secretion of different cytokine patterns (17). Dendritic cells are professional antigen-presenting cells which can initiate primary and secondary T-cell responses (181). Immature dendritic cells originate in the bone marrow and migrate to peripheral non-lymphoid tissues, processing antigen, differentiating to become mature dendritic cells and migrating via the afferent lymph to the draining lymph nodes, where they present antigen to T cells in the T-cell areas (144, 157). Activated/memory T cells travel to the site of inflammation and can be activated at the peripheral sites (99). Recently, a double immunofluorescence study has shown an association between *P. gingivalis* and immature $CD1a^+$ Langerhans cells in the epithelium of gingival sections from periodontitis subjects (36). As immature dendritic cells were limited to the epithelium and mature dendritic cells were restricted to the connective tissue, it was hypothesized that immature dendritic cells could be exposed to *P. gingivalis*, resulting in their activation/maturation and movement into the connective tissue. *In vitro* evidence that dendritic cells internalize *P. gingivalis* was also demonstrated and pulsed dendritic cells could stimulate T cells to proliferate in a dose-dependent manner. *P. gingivalis* sensitization of dendritic cells was further hypothesized to occur in advanced gingivitis or early active periodontitis, followed by the homing of *P. gingivalis*-specific effector T cells with persistent *P. gingivalis* infection in severe periodontitis. Another study has shown that the IL-10 : IL-12 ratio elicited from *P. gingivalis*-pulsed dendritic cells was threefold higher than that from *E. coli*-pulsed dendritic cells. Furthermore, *P. gingivalis*-pulsed dendritic cell-induced proliferation of autologous CD4 cells was lower, as was the release of IFN-γ (90).

Cells other than dendritic cells including macrophages and B cells may initiate secondary immune responses after the induction of major histocompatibility complex class II molecules by cytokines such as IFN-γ (134). Unlike B cells, macrophages and dendritic cells bind antigen by relatively nonspecific mechanisms, so that they may initially activate T cells, while B-cell antigen presentation may induce further activation and clonal expansion of these cells. A recent study has shown a predominance of B cells, rather than macrophages or dendritic cells in periodontitis lesions (71). Furthermore, a study by Mahanonda et al. (119) demonstrated that *P. gingivalis* or *A. actinomycetemcomitans* stimulation of peripheral blood mononuclear cells resulted in upregulation of the costimulatory molecule CD86 and mature dendritic cell marker CD83 on B cells. These activated B cells were potent antigen-presenting cells in mixed leukocyte reactions, stimulating T cells to produce high levels of IFN-γ and minimal IL-5. While this study indicated that B cells induce a Th1 response, other evidence suggests that B cells direct $CD4^+$ T cells to a Th2 pathway (reviewed in 17). In yet another study, when purified populations of monocytes, B cells or dendritic cells presented *P. gingivalis* outer membrane antigens to *P. gingivalis*-specific T cells, the resultant cytokine profiles were consistent with both Th1 and Th2 responses with lower percentages of $IL\text{-}10^+$ T cells, both CD4 and CD8 cells being stimulated to similar degrees (70). Therefore, any shift in the Th1 or Th2 profiles in the periodontal lesion may not be due to the type of antigen presenting cell but due to other factors such as the nature of the antigen. Finally, the low percentages of $IL\text{-}10^+$ T

cells induced by *P. gingivalis* have been suggested to indicate progressive disease such that this cytokine may be of fundamental importance in the control of periodontal disease progression (63).

Suchett-Kaye et al. (182) have suggested that gingival keratinocytes may act as antigen-presenting cells in the progressive lesion. It is already clear that gingival keratinocytes contribute to periodontal disease progression by the secretion of a number of proinflammatory cytokines including IL-1, IL-6 and IL-8 and the expression of adhesion molecules, which aid in the influx of leukocytes into the gingival sulcus. Since *P. gingivalis* and *A. actinomycetemcomitans* can, unlike normal flora, disrupt the gingival epithelial barrier by adhering to and invading epithelial cells and even the connective tissues in diseased sites (47, 166), it is possible that gingival keratinocytes can present *P. gingivalis* and *A. actinomycetemcomitans* antigens to the underlying lymphocytes (162). As well as keratinocytes, other resident cells such as activated endothelial cells and fibroblasts may also play a role in antigen presentation in periodontitis lesions.

Costimulatory molecules

Activation of T cells leading to cytokine production requires a signal transduced through the T-cell receptor as well as a second signal transduced by a costimulatory molecule (56). CD28 is the major costimulatory signal receptor for T cells and its natural ligands are CD80 (B7-1) and CD86 (B7-2), which are expressed either constitutively or after activation on antigen-presenting cells (2). CTLA4 (CD152), a CD28 homolog, is expressed only on activated T cells (48, 108) and, unlike CD28, is a negative regulator of T-cell activation (125). CD152 functions to inhibit T-cell responses and thus has opposing activities to CD28. CTLA-4 binding blocks IL-2 production, IL-2 receptor expression and cell cycle progression (197) and negative regulation of T-cell responses takes place either during the initial triggering of the cells, resulting in tolerance, or during the later stages, to terminate proliferation and effector functions. Where B7 ligands are limited, low levels of CD152 with its greater affinity and avidity for B7 may induce signals which predominate and inhibit T-cell responses. With increased expression of B7, initially on dendritic cells and subsequently on activated B cells, CD28 signals may predominate, resulting in T-cell activation (24).

T cells express CD28 rather than CD152 in periodontal disease tissues (68, 140). $CD28^+$ T cells were found to increase with increasing numbers of B cells, suggesting T-and B-cell interactions with increasing inflammation (68). While *in vivo* studies (68, 140) suggested a limited role for CD152 in periodontal disease, an *in vitro* study reported a higher percent $CD152^+$ CD4 cells in cultures of *P. gingivalis*-stimulated peripheral blood cells from periodontitis patients than from healthy control subjects (5). Anti-CD152 monoclonal antibodies had no effect on the proliferative response of peripheral blood mononuclear cells, although CD152 immunoglobulins did suppress proliferation in the presence of *P. gingivalis*. Orima et al. (140) also correlated the distribution of $B7^+$ cells with that of B cells, and CD86 was found to be the predominant B7 molecule (68). CD80 expression was determined to be expressed mainly by macrophages, whereas both macrophages and B cells were positive for CD86. The very low percentage of positive cells also suggested that the majority of B cells in periodontal disease lesions are not activated in terms of B7 expression (68).

CD28/B7 interactions may influence T-cell cytokine profiles, with Th1 clones being more dependent on B7 than Th2 clones (56). When B7 negative antigen-presenting cells present antigen, Th1 cells are not activated to produce IL-2 and become unresponsive, resulting in downregulation of cell-mediated responses (87). T-cell anergy after transmigration of *A. actinomycetemcomitans*-specific Th1 clones across IFN-γ stimulated major histocompatibility complex class II^+ B7-endothelial cells has been reported (190). In a *Listeria monocytogenes* mouse model, blocking of B7 resulted in a decrease in antigen-specific production of IFN-γ and IL-2 both *in vivo* and by cultured spleen cells (213). Th2 clones, on the other hand, which use IL-4 as their autocrine growth factor, can be activated without CD28 costimulation (124), indicating that activation of differentiated Th2 cells may require other costimulatory molecules (56). However, CD28/B7 binding is necessary to make these cells responsive to IL-4. CD28 increases responsiveness to IL-4 through an IL-1-dependent route, that is, CD28 costimulation is mediated by IL-1 induction in these cells, so that although the mechanism may differ, CD28 is required for activation and proliferation of both Th1 and Th2 cells (124). Yet another report showed that IL-4 production in *Borrelia burgdorferi*-infected BALB/c mice was dependent on CD28/CD86 interaction while CD80/CD86 blockade resulted in expansion of IFN-γ producing T cells, suggesting that other costimulatory pathways may contribute to T-cell

activation during continuous antigen stimulation (176). In another study, injection of *A. actinomycetemcomitans* into the gingiva of rats after the transfer of antigen-specific Th1 clones but not Th2 clones was reported to induce bone resorption. *A. actinomycetemcomitans* lipopolysaccharide induced the expression of both B7-1 and B7-2 on previously negative gingival macrophages and the administration of local or systemic CTLA4Ig, a functional antagonist of CD28 binding to B7, abrogated the bone resorption induced by Th1 cells and gingival challenge with both antigen and lipopolysaccharide. It was suggested that inhibition of B7 expression by this antagonist could prove therapeutic for intervention of inflammatory bone resorption (95).

Innate immunity

Phagocytic cells such as neutrophils and macrophages constitute the first line of defense against bacterial infection. Neutrophils can be found within the gingival sulcus and migrate through the junctional epithelium in all stages of periodontal disease (Fig. 6). In the sulcus, neutrophils form a barrier between the epithelium and plaque (8) which in most cases prevents bacterial invasion of the epithelium and underlying connective tissue (77). However, bacteria such as *P. gingivalis* are able to evade host innate immune responses (reviewed in 38). *P. gingivalis* inhibits the migration of neutrophils from the circulation into the tissues by inhibition of E-selectin expression on endothelial cells (37). *P. gingivalis* also inhibits epithelial cell production of the chemokine IL-8, thus blocking neutrophil transmigration through the oral epithelium (117). Bacteria, including *P. gingivalis,* produce proteases that can cleave complement and immunoglobulins to prevent opsonization and subsequent neutrophil killing of invading bacteria (112, 169, 170, 183). *A. actinomycetemcomitans* produces a protein which inhibits neutrophil chemotaxis and H_2O_2 production (6, 7), and a cytolytic leukotoxin which lyzes susceptible target cells, including neutrophils, monocytes and T cells (121, 185, 186). The ability of the innate immune system to regulate the adaptive immune response has been recognized (175) based on the observation that neutrophils secrete a range of cytokines including IL-1 and the IL-1 receptor antagonist (109).

Macrophages are important mediators of inflammation. On exposure to antigen, macrophages both initiate and enhance the immune response by the secretion of a number of proinflammatory cytokines such as IL-1 and IL-6, T-cell regulating cytokines including IL-10 and IL-12 and a number of chemokines which influence the recruitment of additional monocytes, neutrophils and lymphocytes into the gingival tissues (102). Macrophages also act as antigen-presenting cells in the initial stages of the immune response and play a vital role in the effector stages as microbicidal cells (85). It is difficult to ascertain the regulatory role of macrophages in periodontal disease as few studies have been reported and these have been contradictory. One study

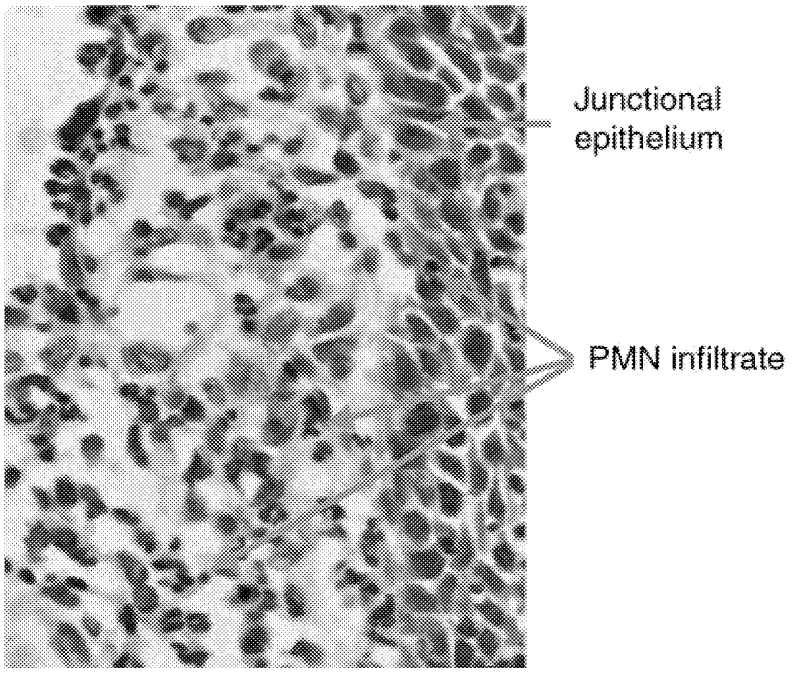

Fig. 6. Neutrophils in the subepithelial tissues and migration through the junctional epithelium.

demonstrated the presence of different subpopulations of macrophages indicative of macrophages at different stages of differentiation and activation, suggesting participation of these cells in the local immune response in periodontal disease (171). However, another study reported no increase in macrophage numbers and little evidence of macrophage activation in advanced periodontitis compared with minimally inflamed tissues (26).

Toll-like receptors

Studies on toll-like receptors are providing answers as to how antigen-presenting cells such as dendritic cells and macrophages recognize bacteria and mount different responses resulting in elimination of invading pathogens (139). Different toll-like receptors recognize different pathogen-associated molecular patterns (194) resulting in the induction of different patterns of immune and inflammatory genes (82). Specificity in the innate immune response may lie in the differences in signal transduction pathways activated by different toll-like receptors (139). A study on *E. coli* lipopolysaccharide and influenza virus, which activate toll-like receptor-4 and toll-like receptor-3, respectively, suggested that a common set of genes was triggered by activating a common signaling pathway, although differences indicated distinct pathways activated by each receptor type (82). *E. coli* lipopolysaccharide and *P. gingivalis* lipopolysaccharide have been shown to exhibit potent toll-like receptor-4 and toll-like receptor-2 agonist activity, respectively, resulting in a differential expression of a panel of genes in murine macrophages. The data supported findings of a shared signaling pathway elicited by toll-like receptor-4 and toll-like receptor-2 agonists that has to diverge, accounting for distinct inflammatory gene expression profiles (79). Activation of dendritic cells by toll-like receptor-4 or toll-like receptor-2 agonists resulted in differences in cytokine and chemokine gene transcription, toll-like receptor-4 stimulation promoting the Th1 cytokine-inducing IL-12p70 and the Th1-associated chemokine IFN-γ inducible protein (IP)-10; toll-like receptor-2 stimulation did not induce IL-12p70 and IP-10 but did induce the IL-12 inhibitory p40 homodimer, favoring Th2 development (152). Injections of *E. coli* or *P. gingivalis* lipopolysaccharide together with OVA induced similar clonal expansion of OVA-specific $CD4^+$ and $CD8^+$ T cells, although while *E. coli* lipopolysaccharide induced a strong IFN-γ response with few or none of the Th2 cytokines IL-5, IL-10 or IL-13, *P. gingivalis* lipopolysaccharide induced the strong Th2 cytokines and lower levels of IFN-γ. *E. coli* lipopolysaccharide also induced IL-12 p70 in CD8 α^+ dendritic cells, while *P. gingivalis* lipopolysaccharide did not, although both types of lipopolysaccharide activated dendritic cells to upregulate costimulatory molecule expression (151). These studies suggest that differential signaling by toll-like receptors may lead to Th1 or Th2 polarization and a lack of toll-like receptor signaling has also been suggested to result in a Th2 response by default (139).

O'Neill (139) suggests that unraveling the mechanisms of toll-like receptor signaling, as well as discovering additional signaling proteins, will increase our understanding of how cells involved in the innate immune response optimize the adaptive immune response, resulting in elimination of the invading microorganism.

Microarrays

Microarray technology incorporates molecular genetics and computer science on an enormous scale to allow investigation of the simultaneous expression of entire genomes to provide information on gene function, disease pathophysiology, disease classification and drug development (74). A major use of this technology is in the identification of associations between genes and cancer (41) and one of the modifications of this new technology is in the use of tissue microarrays (161). These involve the placing of tiny disks of tissue in an array in one recipient paraffin block, enabling the analysis of hundreds of cases simultaneously. This technology allows a highly efficient high-throughput mechanism for the comprehensive characterization of biomarkers of interest (135, 158). Tissue microarrays are proving a powerful tool for the rapid identification of the biological or clinical significance of molecular alterations in tumors (135). Microarray ELISAs have also been reported as a tool that allows the simultaneous analysis of a number of antigens in complex biological samples (89, 203). Immunotyping of leukemias via the concurrent determination of 50 or more CD antigens on leukocytes in a single analysis using a microarray of antibodies has also been reported. This technique compared well with flow cytometry and the intact cells captured on antibody dots allows further characterization using fluorescently labeled antibodies (15). Genes associated with specific biological pathways could be ascertained in periodontal

disease tissues. Microarray analysis of RNA extracted from cells/cell lines *in vitro* could be performed to determine the pathways by which periodontal pathogens induce periodontal destruction. Determination of the genes involved in these pathologic processes and the proteins they produce could lead to assays studying the role of these proteins and ways to downregulate them as a means of immunotherapy.

Conclusion

It is clear that the immunoregulatory control of Th1/Th2 cytokine profiles is fundamental in determining the ultimate outcome of chronic periodontitis. The various mechanisms discussed above are not mutually exclusive and all may play some role. Whether this role is the same in all individuals remains to be determined. In some people, for example, the genetic influence may be the dominant factor; in others, the nature of the antigen (s) may represent the overriding influence. Clearly, the inability to clinically determine the precise nature of the disease process will continue to make research in this area difficult. Nevertheless, because of its fundamental nature, research must continue if susceptibility to periodontal disease is to be understood and tooth loss prevented.

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Shouts and whispers: an introduction to immunoregulation in periodontal disease

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There is little doubt that patient susceptibility to chronic periodontal disease plays a major role in determining the ultimate disease outcome. Susceptibility to periodontal disease involves the interplay between bacteria, the host and environmental factors (Fig. 1). It is well established that bacteria in the dental plaque biofilm are the cause of the inflammation. However, since the 1970s it has been clear that not all plaques result in progressive disease. In 1996 the consensus report of the World Workshop on Clinical Periodontics (1) concluded that three bacterial species, *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* and *Tannerella forsythia*, should be considered as the major periodontal pathogens. Subsequently, Socransky et al. (24) described a number of bacterial complexes with the major 'red complex', consisting of *P. gingivalis*, *Treponema denticola* and *T. forsythia*, being associated with advanced forms of chronic periodontitis. Although there is general support for this concept, it is also well recognized that the presence of the pathogenic bacteria *per se* is insufficient to cause disease. It has recently been shown that there is a high degree of volatility in terms of acquisition and loss of the putative periodontal pathogens over a 5-year period. Relatively few subjects had these organisms on multiple occasions (4). Whether or not the bacteria were actually lost or just declined to levels below the detection limit of the assay remains to be determined. Nevertheless, this study clearly showed the limitations in cross-sectional microbiological studies and periodontal disease and highlights the fact that many individuals may harbor the organisms without manifesting progressive periodontal destruction (4).

Host factors are clearly of fundamental importance (21). In this context, the way in which the host responds to the bacteria is determined by the nature and control of both the innate and adaptive immune responses. As pointed out by Marshall (15) The sulcular and junctional epithelia have been thought to represent the apparent *weak link* in the body's ability to seal out the outside environment i.e. the plaque biofilm. In reality however, it acts more as a gatekeeper, selectively allowing the passage of antigens and cells as well as producing a range of defensive molecules. While the physical barrier function of the epithelium cannot be understated, it is now recognised that epithelia throughout the body produce a diverse range of antimicrobial peptides. To date, at least four families of different antimicrobial peptides (α-defensins, β-defensins, Cathelicidins, Saposins) have been found in humans. An overview of these molecules and their possible role in periodontal disease is presented by Marshall (15) in this issue. This is not an extensive review of defensins but rather an overview to highlight the important contribution of these molecules in disease pathogenesis and as possible therapeutic modalities. For a more comprehensive review, readers are referred to Dale (7).

Once the epithelial barrier with its antimicrobial peptides is breached the adaptive immune response comes into play. Cytokines are central to this response, such that the production of 'appropriate' cytokines results in development of protective immunity and the production of 'inappropriate' cytokines leads to tissue destruction and disease progression (10). Just how the immune system chooses and regulates the right cytokines is unclear, although genetic factors are most likely involved. Recently, Cullinan et al. (3) in a 5-year longitudinal study showed that a specific interleukin (IL)-1 genotype was a contributing but nonessential factor in the progression of periodontal disease. Equally they showed smokers with *P. gingivalis* had significantly more probing depths greater than 3.5 mm compared

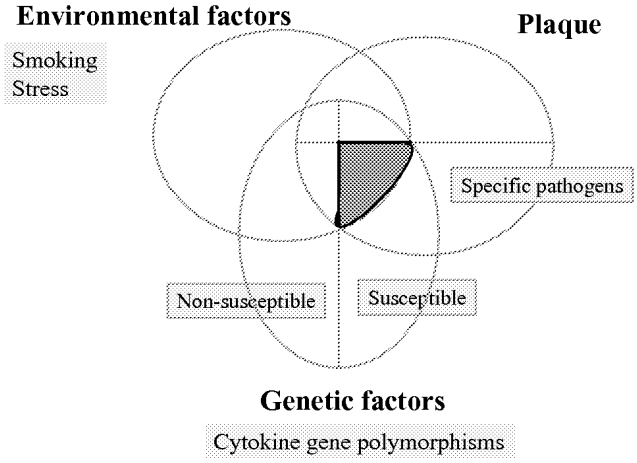

Fig. 1. Susceptibility to periodontal disease.

with smokers without *P. gingivalis*. At the same time, IL-1 genotype-positive smokers had 70% more pockets >3.5 mm than IL-1 genotype-negative smokers (3). This study clearly shows the interplay between bacteria, host and environmental factors.

As stated above, cytokines are central to the pathogenesis of an ever-increasing number of diseases, including periodontal disease. Cytokines are intercellular messengers and as such represent a key mechanism by which cells involved in immune responses communicate. They are usually produced transiently, often in picomolar concentrations, and some, such as IL-4, may have a very restricted range of activity. Indeed, the majority of immune responses occur locally and often between two cells conjugated together (Fig. 2). In this context, the analogy can be drawn that when two cells are talking together they 'whisper'. However, when cells talk to one another at a distance they may 'shout' by producing large amounts of cytokine. Such cytokines such as IL-1 and IL-6 are therefore produced by a large number of cells and are produced in relatively large quantities. It is therefore erroneous to compare the various cytokines quantitatively and suggest that any differences are of greater or lesser biological significance. Within the context of this analogy it can be seen that immunoregulation or control of the immune response in periodontal disease is a series of 'shouts' and 'whispers' as cells talk to one another while they combat the bacterial attack.

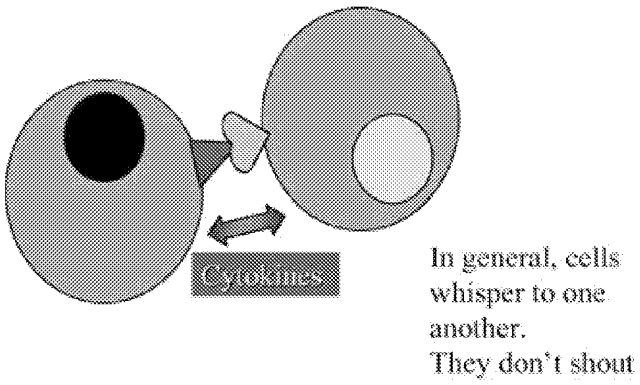

Fig. 2. The majority of immune responses occur locally rather than systemically and often between two cells conjugated to one another.

Cytokines also have a tremendous built-in redundancy, many cytokines having overlapping functions. IL-1 and IL-6 are two such cytokines, as are IL-4 and IL-13. Hence, continuing the 'shouts' and 'whispers' analogy, cells hear the same message from a variety of sources so that the absence of one cytokine but the presence of another may result in the same biological outcome. Equally, many cytokines are antagonistic and again the overall biological effect is the result of the balance between all cytokines rather than their individual levels. It should be obvious therefore that cytokines function as a network and individual elements of the network cannot be studied in isolation (17). For example, Martuscelli et al. (16) have shown that recombinant human IL-11 (an inhibitor of IL-1 production) effectively inhibits periodontal attachment loss in a dog model. Therefore the study of IL-1 without reference to IL-11 (as well as other inhibitors such as IL-10 and IL-1ra) may well be meaningless or at best difficult to interpret. The wealth of new information emerging from the human genome project and related initiatives has revealed many novel cytokines with as yet unknown functions (19). The study of cytokines and their role in immunoregulation and disease pathogenesis will therefore remain a critical element of periodontal research for some years to come.

Since it was first presented at the ICPR meeting in Osaka in 1992 (22) the T helper (Th)1/Th2 hypothesis for the immunoregulation of periodontal disease has attracted a lot of attention (for review see Seymour & Gemmell (23)). A number of studies have shown a decrease in Th1 responses in periodontitis, while others have shown increased Th2 responses. Some studies have claimed a dominance of Th1 response over Th2 responses in periodontitis, whereas others have shown a predominance of Th0 cells in periodontitis (reviewed in 23). These studies are difficult to compare since they used a variety of cells and tissues, a variety of techniques and a variety of stimulants. In addition, the inability to determine disease activity clinically makes interpretation difficult, if not impossible. Nevertheless, it is agreed that control of the Th1/Th2 balance is central to the immunoregulation of periodontal disease.

There is increasing evidence that the Th1/Th2 balance is controlled by a range of factors, all of which are possible within the periodontal tissues. An overview of these factors is presented by Gemmell & Seymour (10) in this issue. These include the following:

The nature of the innate immune response

A strong innate immune response will result in large amounts of IL-12 and IL-18 production by monocytes and neutrophils that, in turn, will promote a Th1 response. Interleukin-18 was discovered in 1989 as INF-γ inducing factor. IL-18 is structurally homologous to IL-1β and, together with their receptors are members of the IL-1R/Toll-like receptor (TLR) superfamily. This causes similar signalling and signal transduction mechanisms. IL-18 is recognized as a cytokine that is able to enhance the maturation of naive T cells to Th1 cells as a cofactor with IL-12, and hence the production of INF-γ. The ability of The importance of IL-18 as co-inducer of INF-γ induction *in vitro* by the successful reduction of INF-γ production in stimulated mouse spleen cells by neutralizing antibodies to murine IL-18. The major role of IL-12 in this mechanism seems to be enhancing IL-18 receptor expression. Interestingly, Th1 cells express IL-18 receptor whereas Th2 do not. Clearly IL-18 could be expected to have a fundamental role in the control of the Th1/Th2 response in periodontal disease. However, the role of IL-18 in periodontal disease and its interplay with IL-12 and indeed IL-15 has not yet been elucidated. Nevertheless the biological activity of IL-18 is reviewed in detail by Delaleu and Bickel (8).

Equally, there is some evidence to suggest that *P. gingivalis* LPS is recognized by Toll-like receptor (TLR)-2 and TLR-6, which promote a Th2 response. Nonpathogenic lipopolysaccharide (e.g. *Escherichia coli*) is recognized by TLR-4 and CD14, which promote a Th1 response. This is discussed in detail by Dixon et al. (9).

The nature of the antigen(s)

As discussed above, it is generally accepted that while different people may respond differently, specific bacteria are nevertheless the cause of periodontal disease. Irrespective of the host response, from a clinical perspective, if a patient has no plaque, they have no disease. In recent years it has also become apparent that complexes of organisms are most probably associated with disease and hence the role of coinfection has become increasingly recognized. Recent data have suggested that combinations of organisms may result in shifts in the Th1/Th2 and antibody profiles (2, 10). In order to understand how combinations of organisms may influence the immune response it is first necessary to have a knowledge of the specific antigens of the pathogenic bacteria. A comprehensive review of these antigens is presented by O'Brien-Simpson et al. (20). These authors point out that each of the periodontal pathogens produce an array of antigens capable of inducing both Th1 and Th2 cytokine profiles and they suggest that multispecies vaccines directed against key bacterial epitopes associated with the acquisition of essential nutrients may restrict proliferation of the pathogens within the biofilm and thereby influence disease progression.

The nature of the antigen-presenting cell

There is ample evidence that the nature of the antigen-presenting cell can determine the nature of the Th1/Th2 profile. Cutler et al. (6) have suggested that the default lesion in periodontal disease is Th1, in which the major antigen-presenting cell is the dendritic cell. At the same time, Gemmell et al. (12) have shown that in periodontitis lesions $CD19^+$, $CD83^+$ B cells are probably the dominant antigen-presenting cell, supporting the concept of shifts in the Th1/Th2 profile between gingivitis and periodontitis. In their review in this issue, Cutler & Jotwani (5) look at the events leading up to antigen presentation by dendritic cells and suggest that as antigen presentation is the limiting step in the generation of an immune response, blocking specific aspects of this could represent a valid strategy for the control of periodontitis.

T-cell receptor (TCR) affinity

There is emerging evidence that high T-cell receptor affinity with short signaling time favors a Th1 response while low T-cell receptor affinity with a prolonged signaling time favors a Th2 response. As yet, however, the T-cell receptor affinity of cells involved in periodontal disease has not been determined. In their review in this issue, Yamazaki & Nakajima (26) raise the possibility of autoimmunity contributing to the periodontal lesion. The expres-

sion of heat shock proteins in the periodontal tissues is presented, together with the possibility that the immune response to their bacterial homolog (GroEL antigens) cross-reacts and hence contributes to the inflammatory lesion. They further present evidence that regulatory T cells, which control autoimmunity, are in fact lacking in the periodontal tissues.

Genetics

Using a mouse model, Gemmell et al. (13) have shown that susceptibility to *P. gingivalis* infection is in part determined by the H2 haplotype and that this also reflects the Th1/Th2 profile. Also, there is increasing evidence that the cytokines which influence Th1/Th2 function are influenced by genetic polymorphism; this may contribute to differences in individual variation in Th1/Th2 responses and therefore susceptibility and progression in periodontal disease. This possibility is reviewed by Taylor et al. (25). These authors point out that while there is no doubt that a genetic element is an essential component of the periodontal lesion, a central role for cytokine gene polymorphisms in immunoregulation remains suggestive.

All of these mechanisms are not mutually exclusive and it is likely that immunoregulation of inflammatory diseases (such as periodontal disease) involve different mechanisms at different times in different patients and probably no one mechanism is more important than another (18). The application of new knowledge and technologies in the fields of genomics, proteomics and structural biology holds real promise in terms of establishing a truly holistic picture of complex diseases as well as providing rational targets for diagnostic and therapeutic strategies. With this in mind, the authors of the series of articles for this issue of *Periodontology 2000* have been charged to describe progress in the complex field of immunoregulation and periodontal disease and to include a discussion of how these novel approaches might enlighten our research efforts in this area. This issue brings together experts from four continents; it does not attempt to reach a consensus but rather to establish our current knowledge and to set the framework for future research.

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Immune Homeostasis and Interferon Status of Newborns from Mothers with Cytomegalovirus and Herpes Simplex Virus Infections.

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Cell mediated and humoral immunity, immunoregulation compartment, phagocyte system and interferon status were analyzed during the first day of life in 96 newborns from mothers with cytomegalovirus and herpes simplex virus infections as well as in 20 newborns from mothers with physiological course of the pregnancy and delivery. As a result of this study the next characteristics were found in newborns of the experimental group: activation of T-compartment of immunity with intense emigration of increased number of early precursor T lymphocytes into the peripheral blood; alteration of the immunoregulation compartment with reduction (or a tendency of reduction) of absolute number of T lymphocytes (CD3(+)), CD4(+) helpers-inducers, the cytotoxic T lymphocyte fraction within CD8(+) cells and increase of the T suppressor fraction; increase of the level of cells, which express receptors for IL-2 (CD25(+)); increase of the number of NK and activated NK (CD16(+)CD8(+)); decrease of the absolute and relative number of mature B lymphocytes (CD20(+)); increase of IgM and IgA synthesis; increased level of the immature forms of neutrophiles and reduced phagocytic ability of some phagocytes; reduction of IFN-ggr; and increase of IFN-alpha level. Thus, cytomegalovirus and herpes simplex virus were shown to affect unfavorably the development of immunity during ontogenesis with alteration of the immune homeostasis, reflecting intrauterine activation of the antigen-specific immune response. All above mentioned may serve as an indication for immunocorrecton therapy, and in particular for IFN preparations with aim to correct immune and IFN deficiency among such children immediately after their birth.

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